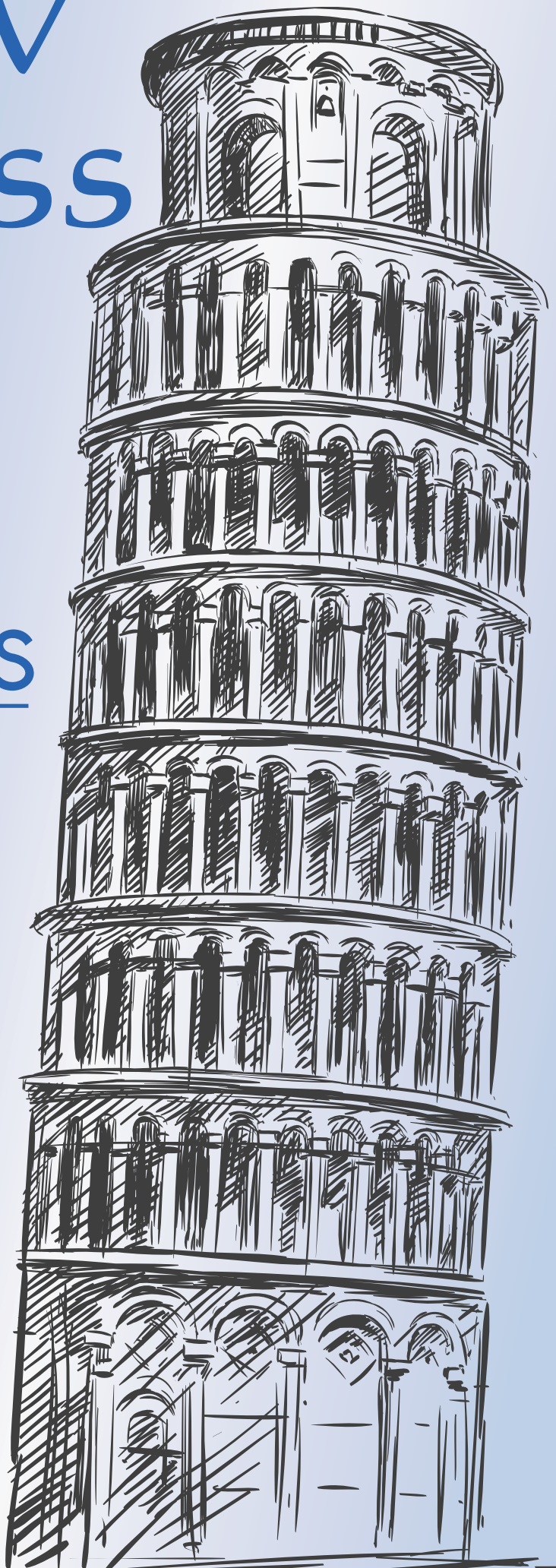
XIII FISV Congress

AAI - ABCD - AGI - SIB - SIBBM - SIBE - SIBV - SIC
SICA - SIF - SIGA - SIMA - SIMGBM - SIP - SIPaV



PROGRAM and ABSTRACTS

Pisa, Italy
September 24-27, 2014

FISV Federazione Italiana Scienze della Vita

www.fisv.org
www.fisv2014.azuleon.org

FISV - Federazione Italiana Scienze della Vita

Program and Abstracts of the XIII FISV CONGRESS

Palazzo dei Congressi, Pisa, Italy

September 24 - 27, 2014

CONTENTS

WELCOME LETTER

It is a great pleasure to welcome you to the 13th National Congress of the Italian Federation of Life Sciences (FISV).

This is the second Congress that I have the honour to chair, and follows the Congress held two years ago at La Sapienza University, which had a relevant scientific success as well as a large participation of attendees, especially young scientists. I am glad that the successful trend of participation continues this year in Pisa. The interest of the Italian biologists for our National Congress and their enthusiastic response to the activities of FISV is growing and consolidates the impact of the Federation on the Italian Scientific Community. I am sure that this will continue in the future years. The Federation welcomes this year a new member, the prestigious Society of Biochemistry (SIB), which joined the Federation last year. The Federation represents now a total of 15 Scientific Societies and about 10000 scientists involved in biological and biomedical research. All 15 Societies actively contributed to the Congress organization. The program covers topics at the forefront of modern biology and offers contributions on emerging issues from some of the best laboratories in the World. Four plenary lectures, three plenary symposia and six parallel symposia will host outstanding speakers. Mini-symposia, covering all the interests of the different Societies of the Federation, will highlight some of the best contributions chosen among the submitted abstracts.

I warmly thank the Societies of the Federation, the Organizing Committee and the Organizing Secretariat, which have greatly contributed to the success of this event. I also thank all of you for choosing to be in Pisa with us this year.

Best wishes,
Il Presidente della FISV
Prof. Felice Cervone

MEMBER SOCIETIES

FISV - Federazione Italiana Scienze della Vita

Italian Federation of Life Sciences

AAI	**Associazione Antropologia Italiana**
ABCD	**Associazione di Biologia Cellulare e del differenziamento**
AGI	**Associazione Genetica Italiana**
SIB	**Società Italiana di Biochimica e Biologia Molecolare**
SIBBM	**Società Italiana di Biofisica e Biologia Molecolare**
SIBE	**Società Italiana di Biologia Evoluzionistica**
SIBV	**Società Italiana di Fisiologia Vegetale**
SIC	**Società Italiana di Cancerologia**
SICA	**Società Italiana di Chimica Agraria**
SIF	**Società Italiana di Farmacologia**
SIGA	**Società Italiana di Genetica Agraria**
SIMA	**Società Italiana di Mutagenesi Ambientale**
SIMGBM	**Società Italiana di Microbiologia Generale**
SIP	**Società Italiana di Patologia**
SIPaV	**Società Italiana di Patologia Vegetale**

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Giovanni Vannacci, (Pisa)

Organising Secretariat

Marina Nobilio
Lucia Tufano
Lucia Ugo
Via dei Sardi, 70
00185 Roma
e-mail: segreteria@fisv.org

FISV Secretariat

Marina Nobilio
Via dei Sardi, 70
00185 Roma
e-mail: segreteria@fisv.org

FISV 2014 Web Services

Azuleon Meetings
Paulo Magalhães
Elena Papinutto
email: magalhaes@azuleon.com
elena.papinutto@azuleon.org
http://meetings.azuleon.org

UNDER THE AUSPICES OF

Università di Pisa

Scuola Superiore Sant'Anna

SESSION ORGANISERS

Plenary Lecture

Felice Cervone
Genaro Ciliberto
Paolo Trost
Alessandro Vitale

Plenary Symposia

Genaro Ciliberto
Bianca Colonna
Bruno Giardina
Milena Grossi
Ruggero Pardi
Giancarlo Solaini

Parallel Symposia

Antonio Antoccia Conicella
Margherita Bignami
Domenico Carputo
Vito De Pinto
Giovanni Destro Bisol
Maurizio Gatti
Matteo Lorito
Anna Moroni
Roberto Pinton

Mini Symposia

Lucia Altucci
Elena Battaglioli
Marialina Bernardini
Giorgio Bertorelle
Marco Bianchi
Marzia Bianchi
Silvia Bonaccorsi
Tiziana Bonaldi
Francesco Bonomi
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Sergio Tofanelli
Antonio Torroni
Mario Ventura
Paolo Visca

PROGRAM

Wednesday, September 24

11:00 - 13:00 **Registration**

13:30 - 14:00 **Opening**

Felice Cervone *(FISV President)*
Lucio Luzzatto *(FISV past-President)*

Edoardo Boncinelli
Commemoration of Ferruccio Ritossa

14:00 - 15:00 **Plenary Lecture** (EMBO Lecture)

Giacomo Cavalli *(Montpellier, France)*
Genome regulation by Polycomb proteins and 3D chromosome folding

15:00 - 17:00 **Plenary Symposium**

Microorganisms and Cancer

Chairs: Bianca Colonna *(Sapienza, University of Rome)* Milena Grossi *(Sapienza, University of Rome)*

Teresa Frisan *(Stockholm, Sweden)*
Carcinogenic properties of bacterial genotoxins

Marina de Bernard *(Padua)*
Helicobacter pylori infection and Th17 profile in chronic gastritis

Massimo Tommasino *(Lyon, France)*
The role of human papillomaviruses in carcinogenesis

Massimo Levrero *(Rome)*
Molecular mechanisms of HBV-associated hepatocarcinogenesis

17:30 - 19:00 **Round Table**

La Tracciabilita Genetica per uomini, animali e piante: aspetti scientifici, economici, giuridici ed etici

Chairs: Walter Daviddi

Tenente Colonnello **Giampietro Lago** *(PhD, comandante del R.I.S. di Parma)*
Tracciabilità umana

Prof. **Andrea Armani** *(Responsabile Fish Lab e Docente di Ispezione Alimenti di origine Animale, Università di Pisa)*
Tracciabilità animali

Prof. **Luigi Frusciante** *(Ordinario di Genetica Agraria, Università di Napoli Federico II)*
Tracciabilità vegetali

Prof. **Gianluca Brunori** *(Economista, Ordinario di Economia Agraria - Docente di Bioeconomia, Università di Pisa)*
Aspetti economici

Prof. **Sergio Bartolommei** *(Filosofo e docente di Bioetica - Università di Pisa)*
Aspetti etici

Thursday, September 25

09:00 - 10:00 Plenary lecture

Elena Cattaneo *(Milan)*
Huntington, the story of an ancient gene in search of a better future

10:00 - 10:30 Coffee break

10:30 - 12:30 Plenary Symposium

Modeling cell pathophysiology by integrative network-based association studies

Chairs: Gennaro Ciliberto *(Istituto Nazionale Tumori, Naples)* Ruggero Pardi *(UniSr, Milan)*

Andrea Califano *(New York, NY, USA)*
Elucidating non oncogene dependencies in cancer by assembly and interrogation of regulatory networks

Gianni Cesareni *(Rome)*
The logic network underlying the anti-cancer activity of metformin: a systems approach

Diego Di Bernardo *(Naples)*
Networking drugs and diseases: a systems biology perspective

Participant's Short talk

Graziano Martello *(Padua)*
Cracking the naive Pluripotency code

Ermanno Rizzi *(Vimercate)*
Genomic Integration Sites (IS) analysis of virus-based vectors for gene therapy: the Integrome

Davide Corona *(Palermo)*
Role of the chromatin remodelling factor ISWI intTissue regeneration

12:30 - 14:30 Lunch and Posters viewing

Mini Symposia Session I

14:30 - 15:45 1. Metabolism and its regulation in health and diseases

Chairs: Paola Chiarugi *(University of Florence)* Loredano Pollegioni *(Università degli Studi dell'Insubria)*

Santina Bruzzone *(Genoa)*
Impaired increase of plasma abscisic acid in response to oral glucose load in type 2 diabetes and in gestational diabetes

Paolo Cirri *(Florence)*
Fibroblasts transfer lipids and proteins to cancer cells through cargo vesicles supporting tumor growth

Erika Fiorino *(Milan)*
Histone deacetylases (HDACs) and cholesterol catabolism: effect of HDAC7 deletion on lipid and lipoprotein profile

Paolo Porporato *(Brussels, Belgium)*
A mitochondrial switch promotes tumor metastasis

15:45 - 17:00 **8. Nutrition Biochemistry**

Chairs: Francesco Bonomi *(University of Milan)* Silvana Hrelia *(University of Bologna)*

Cristina Angeloni *(Bologna)*
Neuroprotective effects of sulforaphane on methylglyoxal-induced glycation in SH-SY5Y cell line

Maria Cristina Mezzacapo *(Naples)*
Nutritional values and radical scavenging capacities of lentil (*Lens culinaris* Medik) seeds in Valle Agricola district, Italy

Gianna Ferretti *(Ancona)*
Protective effect of apple polyphenols on methylglyoxal-induced glycation of human High density lipoproteins

Stefania Iametti *(Milan)*
Physiological consequences of beta-lactoglobulin adsorption on hydrophobic surfaces: making a bad food allergen even worse?

Paola Antonia Corsetto *(Milan)*
Docosahexaenoic acid influences cholesterol metabolism in cancer cells

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14:30 - 15:45 **3. Genomics, Proteomics and System Biology**

Chairs: Tiziana Bonaldi *(Istituto Europeo di Oncologia)* Manuela Helmer-Citterich *(University of Rome, Tor Vergata)*

Federica Ciregia *(Pisa)*
Glucagon-like peptide-1 protects INS-1E against palmitate-mediated β-cell dysfunction: a proteomic study

Marco Fondi *(Florence)*
Modelling in the cold: genome scale metabolic reconstruction and constraints-based modelling of the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125

Alessio Mengoni *(Florence)*
Evolution of regulatory networks in *Sinorhizobium meliloti* is species- and replicon-specific

Angela Messina *(Catania)*
The interactome of human VDAC3 revealed in HeLa cells by affinity purification tag technique

Maria Manuela Rigano *(Naples)*
Improvement of the nutritional potential of tomato fruits through pyramiding of favorable alleles

15:45 - 17:00 **23. Transcription Mechanisms and Networks**

Chairs: Duccio Cavalieri *(Fondazione Edmund Mach)* Rodolfo Negri *(Sapienza, University of Rome)*

Cecilia Battistelli *(Rome)*
DNMT3s and miRs-29 in EMT/MET dynamics: a role for HNF4α

Sara Borghi *(Bologna)*
A novel regulatory switch controls the expression of *Neisseria meningitidis* NHBA at physiologically relevant temperatures

Valentina Cappelletti *(Trento)*
Multicellularity in yeast: heat shock proteins as actors of an epigenetic regulatory network

Angela Cirigliano *(Rome)*
Impairment of the COP9 signalosome in yeast leads to activation of autophagy

Silvia Santopolo *(Rome)*
The Dof protein DAG2 is a positive regulator of the phyB-mediated seed germination process

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14:30 - 15:45 **10. Human Genetic and Genomic Diversity**
Chairs: Antonio Torroni *(University of Padua)* Mario Ventura *(University of Bari)*

Lucia Pellè *(Pisa)*
A case-control association study suggests a role for CYP2E1 in the susceptibility to thyroid carcinoma

Andrea Quagliariello *(Bologna)*
Exploring genetic variability of genes involved in nutrition and thermoregulation processes in european populations

Carlo Sidore *(Cagliari)*
Whole genome sequencing of 3,514 individuals from the founder population of Sardinia

Roberta Rosa Susca *(Ferrara)*
Y-chromosome and mtDNA diversity in the context of Eurasian language diversity

Beniamino Trombetta *(Rome)*
Phylogenetic refinement and SNP-based dating of human MSY haplogroup E

15:45 - 17:00 **12. Evolution**
Chairs: Sergio Tofanelli *(University of Pisa)* Giorgio Bertorelle *(University of Ferrara)*

Elena Bitocchi *(Ancona)*
Evolutionary history of common bean (*Phaseolus vulgaris* L.)

Irene Cardinali *(Perugia)*
The horse mitochondrial haplogroup variation in ten Italian local breeds

Claudia Catacchio *(Bari)*
Gorilla centromere DNA: an answer to longstanding questions?

Chiara Papetti *(Bremerhaven, Germany)*
Population genetics of a pelagic notothenioid fish along the Antarctic Peninsula

Claudia Vannini *(Pisa)*
Symbiosis evolution: retracing the tangled path from establishment to obligate association in the *Betaproteobacteria-Euplotes* relationship

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14:30 - 15:45 **9. Epigenetics and epigenetic Therapies**
Chairs: Lucia Altucci *(Seconda Università di Napoli)* Davide Corona *(Istituto Telethon Dulbecco, Palermo)*

Fabio Coppedè *(Pisa)*
Genetic and environmental factors linked to DNA methylation in colorectal cancer revealed by artificial neural networks

Svetlana Danovska *(Rome)*
In vivo selection of JARID histone demethylases inhibitors and their use to enlighten the biological role of these enzymes in yeast and mammalian cells with focus on transcriptional regulation

Maria Denaro *(Pisa)*
Genetic and epigenetic implications of the MTHFR gene in cardiovascular disease

Stefania Sarno *(Padua)*
Analysis of the interaction between LSD1, lysine-specific demethylase 1, and protein kinase CK2

Andrea Stoccoro *(Pisa)*
Gene specific DNA methylation analysis in peripheral blood as potential biomarker for Alzheimer's Disease

15:45 - 17:00 **24. Non coding RNA**
Chairs: Elisa Caffarelli *(IBPM, CNR Roma)* Massimiliano Pagani *(Ist. Nazionale Genetica Molecolare, Milan)*

Valeria Bevilacqua *(Rome)*
A novel long noncoding RNA promoting neural induction of human induced pluripotent stem cells

Flavia Biamonte *(Catanzaro)*
MiR-21 over-expression in K562 cells silenced for ferritin heavy chain (FHC) is mediated by oxidative stress

Ilaria Laudadio *(Rome)*
RNA interference determines nucleosome occupancy at human Transcription Start Sites by interacting with SWI/SNF complex

Ilaria Panzeri *(Milan)*
LincRNAs landscape in human lymphocytes highlights regulation of T cell differentiation by linc-MAF-4

Alberto Passi *(Varese)*
O-GlcNAcylation induces hyaluronan synthase 2 transcription modulating promoter chromatin structure via long non coding RNA

14:30 - 15:45 **15. Oncogenes and Tumor suppressors**
Chairs: Milena Grossi *(Sapienza, University of Rome)* Alberto Inga *(University of Trento)*

Paolo Armando Gagliardi *(Candiolo)*
PDK1-mediated activation of MRCKα regulates directional cell migration and lamellipodia retraction

Alberto Inga *(Trento)*
The 5'-untranslated region of $p16^{INK4a}$ melanoma tumor suppressor acts as a cellular IRES, controls mRNA translation during hypoxic stress, and is a target of YBX1

Alessandra Pollice *(Naples)*
MDM2-mediated degradation of p14ARF: a novel mechanism to control ARF levels in cancer cells

Maria Cristina Sorrentino *(Naples)*
Epigenetic role of the N-MYC/LSD1 complex in Neuroblastoma

Annaelena Troiano *(Naples)*
YB-1 and ΔNp63α cross-talk in the control of squamous carcinoma cell adhesion and survival

15:45 - 17:00 **22. Environmental and Molecular Mutagenesis**
Chairs: Lucia Migliore *(University of Pisa)* Sofia Pavanello *(University of Padova)*

Silvestro Conticello *(Florence)*
The RNA editing enzyme APOBEC1 induces somatic mutations and its mutational signature is present in esophageal adenocarcinomas

Stefania De Marco *(Perugia)*
Investigation of the cytotoxic, genotoxic and apoptotic activities of *trans*-cinnamaldehyde: pure compound and botanical matrix (cinnamon tea)

Eugenia Dogliotti *(Rome)*
Genetic and environmental influence on the activity of the DNA repair enzyme OGG1: a twin study

Giada Frenzilli *(Pisa)*
Potential use of the isoflavonic phytoestrogen genistein in thyroid cancer therapy

Chiara Uboldi *(Pisa)*
A panel of tests to evaluate genotoxicity and carcinogenicity induced in vitro by metal oxide nanoparticles

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14:30 - 15:45 **17. Immunology and Host-Pathogen Interaction**

Chairs: Paolo Visca *(University of Rome 3)* Marialina Bernardini *(Sapienza, University of Rome)*

Gianluca Baldanzi *(Novara)*
Inhibition of Diacylglycerol kinase alpha rescues TCR-induced diacylglycerol signaling and restimulation induced cell death in XLP lymphocytes

Anna-Karin Hermansson *(Rome)*
Shigella flexneri induced cell death in human monocyte derived dendritic cells

Lisa Lombardi *(Pisa)*
Molecular characterization of a *Candida orthopsilosis* putative adhesin

Valeria Sgambati *(Naples)*
ApoE-AP, a new human antimicrobial peptide with promising anti-inflammatory properties

Marina Zoppo *(Pisa)*
Targeted gene disruption to investigate the role of *Candida parapsilosis* putative adhesins

15:45 - 17:00 **18. Plant Development and Disease**

Chairs: Simone Ferrari *(Sapienza, University of Rome)* Giuseppe Firrao *(University of Udine)*

Alessandra Boccaccini *(Rome)*
The Arabidopsis DAG1 protein plays a key role in regulating hormonal balance during seed development

Andrea Carucci *(Rome)*
Members of *AtCuAO* gene family exhibit different tissue-and organ-specific expression patterns during seedling development and distinct responses to hormone or stress treatments

Simone Luti *(Florence)*
PAMP activity of cerato-platanin: a proteomic study

Maria Benedetta Mattei *(Rome)*
Proteomic insights into oligogalacturonide signalling in plant defence and development

Maurizio Trovato *(Rome)*
Dissecting the role of proline in pollen development

17:00 - 17:30 **Coffee break**

17:30 - 20:00 **Societies'time**

AGI

17:30 - Premio Dottorato AGI/Zanichelli 2014 e presentazione del lavoro premiato

18:00 - Assemblea dei Soci AGI

SIB

Assemblea dei Soci

Consegna Premio SIB

Consegna Premio Heritage

Votazioni per il parziale rinnovo del C D

SIBV

17:30 - Lecture e consegna del premio Baccarini Melandri

18:30 - Assemblea dei soci

SIMA

17:30 - Premiazione dei migliori poster

18:00 - Assemblea straordinaria dei Soci SIMA

SIMGBM

17:30 - Paolo La Colla (Univ. Cagliari), "Dalle cefalosporine agli antivirali"

18:00 - Consegna Premi e Presentazioni:

- Premio Naicons
- Premio Novartis
- Premi Tatò e SIMGBM

19:00 - Assemblea Ordinaria della Società

SIBE

Discussione sul convegno SIBE2015 a Bologna

Consegna borse di viaggio e premio per miglior articolo 2013-2014 in biologia evoluzionistica

Talk del vincitore del premio

Comunicazioni dalla SIBE

20:30 **Social dinner**

Chiostro Chiesa Santa Maria del Carmine

Friday, September 26

09:00 - 11:00 Plenary Symposium

Metabolic changes in tumor cells

Chairs: Giancarlo Solaini *(University of Bologna)* Bruno Giardina *(Università del Sacro Cuore, Rome)*

Muller Fabbri *(Los Angeles)*
MicroRNAs as master regulators of the biology of the tumor microenvironment

Paolo Bernardi *(Padua)*
Between life and death: a dual function for the mitochondrial F-ATP synthase

Alessandra Baracca *(Bologna)*
Role of the inhibitor protein (IF1) of the mitochondrial ATP synthase on tumor cells metabolism

Paola Chiarugi *(Florence)*
OXPHOS and PKM2 within tumor-stroma interplay: a new druggable synergy

Roberto Scatena *(Rome)*
The cancer stem cells hypothesis claims for a re-evaluation of cancer metabolism

11:00 - 11:30 Coffee break

11:30 - 13:30 Parallel Symposia

1. The multiple facets of genome instability

Chairs: Margherita Bignami *(ISS , Rome)* Antonio Antoccia *(Roma Tre University)*

Stéphanie Tomé *(Paris)*
Triplet repeat instability and human disorders

Daniela Cimini *(Blacksburg, USA)*
Causes and effects of karyotype alterations

Antonio Musio *(Pisa)*
Mutant cohesin drives chromosomal instability in early colorectal adenomas

Francesca Demichelis *(Trento)*
The dynamics of cancer genomes during disease progression

2. Epigenetics: disturbance or resource for plant improvement?

Chairs: Clara Conicella *(IBBR, Portici-Naples)* Domenico Carputo *(University of Naples)*

Marie Mirouze *(Montpellier, France)*
The epigenetic guardians of genome stability in plants

Pasquale Termolino *(Portici)*
Histone acetylation and sex-specific recombination variation in Arabidopsis thaliana

Serena Varotto *(Padua)*
Epigenomics of stress response in maize leaf

Emanuele De Paoli *(Udine)*
Challenged from inside and outside: plant epigenetic responses to genomic modifications and environmental cues

Emidio Albertini *(Perugia)*
Use of MSAP markers to analyze the effects of abiotic stresses on DNA methylation

3. Biochemistry of intracellular channels, carriers and pores

Chairs: Vito De Pinto *(University of Catania)* Anna Moroni *(University of Milan)*

Vito De Pinto *(Catania)*
Introduction: distinct biochemical features and functional roles of VDAC isoforms of outer mitochondrial membrane

Tatiana Rostovtseva *(Bethesda, MD, USA)*
Role of VDAC in regulating mitochondrial respiration in cancer cells and in neurodegenerative disease

Gerhard Thiel *(Darmstadt, Germany)*
Using small viral ion channels as a tool to understand protein sorting

Ildiko Szabo *(Padova)*
Potassium channel(s) in the inner mitochondrial membrane

Giuseppe Fiermonte *(Bari)*
The biochemical function of uncoupling protein 2, unknown until now

Cesare Indiveri *(Cosenza)*
Cation transporters in health and disease: novel functions and role as drug targets

13:30 - 15:30 Lunch & Posters viewing

15:30 - 16:30 Plenary Lecture

Stephen Howell *(Ames, IA, USA)*
The unfolded protein response in plants — facing a changing world

16:30 - 17:00 Coffee break

Mini Symposia Session II

17:00 - 18:15 2. Cellular stress, apoptosis and autophagy

Chairs: Paola Fortini *(ISS, Rome)* Gian Maria Fimia *(University of Salento)*

Silvia Cetrullo *(Bologna)*
3-hydroxytyrosol protects chondrocytes against microRNA-9 increased by oxidative stress

Giorgia Foggetti *(Genoa)*
Induction of autophagy by mutant p53-targeting molecules in cancer cells

Lorenza Garribba *(Rome)*
The fine tuning of autophagy and differentiation in the skeletal muscle

Daniele Lombardo *(Messina)*
Crosstalk between autophagy and apoptosis during HSV-1 replication: possible role of Us11 viral protein

Cristina Mazzoni *(Rome)*
mRNA degradation and its effect on cellular lifespan and apoptosis in yeast

18:15 - 19:30 21. Protein Synthesis, Degradation and Homeostasis

Chairs: Marzia Bianchi *(University Carlo Bo, Urbino)* Paola Londei *(Sapienza, University of Rome)*

Daniela Braconi *(Siena)*
HGA-induced aggregation and fibrillogenesis of amyloidogenic proteins: implications in alkaptonuria

Rita Del Giudice *(Naples)*
Structural perturbations in hereditary amyloidosis: the case of two variants of apoA-I

Maria Elena Miranda Banos *(Rome)*
A single-chain variable fragment antibody against Z alpha1-antitrypsin prevents intracellular polymerisation

Emanuela Pedrazzini *(Milan)*
Proteostasis of the A. thaliana TPK/KCO channels

Alessandro Vitale *(Milan)*
An evolutionary model for protein body formation in the endoplasmic reticulum of cereal endosperm cells

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17:00 - 18:15 **6. Development, Differentiation and Aging**

Chairs: Marco Bianchi *(DIBIT, Milan)* Giorgio Camilloni *(Sapienza, University of Rome)*

Vittorio Abbonante *(Pavia)*
Megakaryocytes express extracellular matrix components

Nicoletta Filigheddu *(Novara)*
Unacylated ghrelin promotes skeletal muscle regeneration

Liliana Felicia Iannucci *(Naples)*
Silencing of Drosophila H/ACA snoRNP pseudouridine synthase dysregulates key developmental pathways

Giuseppe Lupo *(Rome)*
Epigenetic regulation of positional identity in neural progenitors

Robert Vignali *(Pisa)*
Hmga2 is required for the neural crest cell EMT and migration in the Xenopus embryo

18:15 - 19:30 **16. Stem Cells, IPSCs, Cancer Stem Cells**

Chairs: Giuseppe Lupo *(Sapienza, University of Rome)* Eugenia Dogliotti *(ISS, Rome)*

Daniela Capello *(Novara)*
Diacylglycerol kinase alpha contributes to tumorigenicity and invasiveness of cancer stem cells

Simona Daniele *(Pisa)*
Role of adenosine receptors in survival and differentiation of glioblastoma stem cells

Elisa Paolicchi *(Pisa)*
Role of Polycomb/HIF/VEGF pathway in the etiology of mesothelioma

Maria Trincavelli *(Pisa)*
Allosteric modulation of A2B adenosine receptors favours mesenchymal stem cell differentiation to osteoblasts

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17:00 - 18:15 **7. Environmental Microbiology and Biotechnology**

Chairs: Margherita Sosio *(NAICONS, Saronno)* Roberto De Philippis *(University of Florence)*

Alessandra Adessi *(Florence)*
Understanding the role of EPSs in BSCs: study of the physiological response of the phototrophic community to EPS removal

Alice Checcucci *(Florence)*
Exploring the genetic basis con competition in bacterial symbionts: the acdS gene in the rhizobium *Sinorhizobium meliloti*

Luca Dall'Osto *(Verona)*
Domestication of the green alga *Chlorella sorokiniana*: reduction of antenna size improves light-use efficiency in a photobioreactor

Chiara Schiraldi *(Naples)*
Biotechnological transformation of hydrocortisone to prednisolone by coupling *Streptomyces roseochromogenes* and *Arthrobacter simplex* biocatalytic activities

Giulia Spini *(Florence)*
Phenotypic variation of the symbiotic nitrogen-fixing bacterium *Sinorhizobium meliloti* in response to the plant flavonoid luteolin investigated by Phenotype Microarray

18:15 - 19:30 **11. Genetic of Microorganisms**
Chairs: Davide Roncarati *(University of Bologna)* Annamaria Puglia *(University of Palermo)*

Emanuele Bosi *(Florence)*
MeDuSa: a multi-draft based scaffolder

Rosaria Campilongo *(Rome)*
Towards the characterization of the role of YnfB protein in *E. coli*

Alberto Danielli *(Bologna)*
Helicobacter pylori acid acclimation: ménage à trois at the complex arsR promoter

Andrea Moglia *(Turin)*
High yield production of avenanthramide analogous endowed with antioxidant properties

Francesco Pini *(Villeneuve d'Ascq, France)*
Cell cycle regulation by CtrA in *Sinorhizobium meliloti*

17:00 - 18:15 **4. Chromosome Biology, Cell Division and Cell Cycle**
Chairs: Silvia Bonaccorsi *(Sapienza, University of Rome)* Antonio Musio *(CNR, Pisa)*

Ivana Colao *(Messina)*
Involvement of ERK1/2 in cell cycle regulation during Herpes Simplex Virus replication

Giulia Guarguaglini *(Rome)*
Aurora-A kinase inhibition: mitotic effects and implications for anti-cancer strategies

Linda Mannini *(Pisa)*
SMC1B, a new mitotic-specific cohesin component, is involved in gene expression

Alice Mazzagatti *(Pavia)*
The sliding behaviour of horse chromosome 11 centromere

Isabella Saggio *(Rome)*
AKTIP, an E2 variant enzyme that interacts with lamin, is required for correct telomere maintenance

18:15 - 19:30 **5. DNA replication, Repair and Recombination**
Chairs: Marco Muzi Falconi *(University of Milan)* Pietro Pichierri *(ISS, Rome)*

Francesca Grasso *(Rome)*
Colorectal carcinogenesis and the inflammatory response: a role for the MUTYH DNA glycosylase

Antonello Lorenzini *(Bologna)*
A cell culture comparative biology approach to study mechanisms of genome stability and their relevance for species longevity: a newer interpretation of DNA damage induced nuclear foci

Valentina Palermo *(Rome)*
WRN phosphorylation by CDK modulates pathway choice to repair DNA double strand breaks after end-resection

Martina Stevanoni *(Padua)*
Altered replication timing of the frataxin gene in the presence of the GAA/TTC repeat expansion

Micol Tillhon *(Pavia)*
CBP/p300-mediated acetylation of PCNA is required for its chromatin removal and degradation in nucleotide excision repair

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17:00 - 18:15 **13. Neurobiology**

Chairs: Tommaso Pizzorusso *(CNR Pisa)* Elena Battaglioli *(Università degli Studi di Milano)*

Santina Cutrupi *(Turin)*
Estrogen regulates neuroglobin transcription in neuronal and non-neuronal cancer cells

Cecilia Mannironi *(Rome)*
miR-135a and the regulation of neuronal plasticity under stress conditions

Debora Napoli *(Pisa)*
Experience-dependent DNA methylation regulates plasticity in the developing visual cortex

Luigi Palmieri *(Bari)*
Mutations in the mitochondrial citrate carrier SLC25A1 are associated with impaired neuromuscular transmission

18:15 - 19:30 **14. Cell Communication, Cell Adhesion and Membrane Trafficking**

Chairs: Santina Bruzzone *(University of Genova)* Luca Primo *(Università of Torino)*

Valentina Bettio *(Novara)*
Diacylglycerol kinase alpha regulates mitotic spindle orientation during epithelial morphogenesis

Eleonora Da Pozzo *(Pisa)*
Synthetic agents for protein dimerization, a bridge between chemistry and biology for protein characterization and potential drug development.

Laura di Blasio *(Turin)*
PDK1 regulates focal adhesion disassembly through modulation of αvβ3 integrin endocytosis

Nunzia Di Maio *(Naples)*
Dyskerin depletion perturbs cell migration and adhesion in colon cancer cells

Tiziana Vigliarolo *(Genoa)*
Abscisic acid transport in human erythrocytes

17:00 - 18:15 **19. Plant Metabolism and Environmental Stress**

Chairs: Francesco Licausi *(Scuola Superiore S.Anna, Pisa)* Anna Maria Ranieri *(University of Pisa)*

Francesca Verrillo *(Varese)*
Proteome of *Triticum aestivum* cv Bologna affected by free – air CO_2 enrichment

Maria Concetta de Pinto *(Bari)*
Involvement of DNA methylation in the control of cell growth during heat stress

Stefania Pasqualini *(Perugia)*
Plant response mechanisms to drought and insect pest attack: signals from the roots to the leaves

Cristina Sudiro *(Padua)*
Addressing molecular and cellular response mechanisms induced by salt stress in two contrasting salt sensitivity varieties of Italian rice (*Oryza sativa* ssp. Japonica)

Mirko Zaffagnini *(Bologna)*
Unraveling the redox regulation and structure of Calvin-Benson cycle enzymes from *Chlamydomonas reinhardtii*: from proteomic data to in vitro studies

18:15 - 19:30 **20. Plant Nutrition**
Chairs: Luca Espen *(University of Milano)* Maurizio Chiurazzi *(CNR Napoli)*

Moez Maghrebi *(Milan)*
Sentinel plants to improve sulfur use efficiency: living instruments for nondestructive analysis

Bhakti Prinsi *(Milan)*
Modulation of root glutamine synthetase isoforms and plant amino acid balance in response to nitrogen nutrition in maize

Vladimir Totev Valkov *(Naples)*
Systematic characterization of members of the *L. Japonicus* NPF family up-regulated in symbiotic nitrogen fixation nodules. What's their role on nodule functioning?

Laura Zanin *(Udine)*
Transcriptional and physiological aspects of Fe deficiency response in roots of *Zea mays*

Michela Zottini *(Padua)*
Plant growth promotion effects of endophyte community associated to *Vitis vinifera* cv. Glera

Saturday, September 27

09:00 - 11:00 Parallel Symposia

4. Telomeres in model organisms: similarities and differences with human telomeres

Chair: Maurizio Gatti *(Sapienza, University of Rome)*

Maurizio Gatti *(Rome)*
Introduction

Maria Pia Longhese *(Milan)*
Protection of telomeres: mechanisms and regulation in S. cerevisiae

Giovanni Cenci *(Rome)*
Organization and evolution of Drosophila telomeres

Miguel Godinho Ferreira *(Oeiras, Portugal)*
Zebrafish Telomerase is required for normal lifespan and cancer

5. Plant-soil relationships as key factors for food security and sustainability

Chairs: Roberto Pinton *(University of Udine)* Matteo Lorito *(University of Naples)*

Paolo Nannipieri *(Florence)*
Omics approaches to unravel plant-soil relationships

Pascal Simonet *(Lyon, France)*
Soil Metagenomics: state of the art/The role of bacterial transformation in the rhizosphere

Matteo Lorito *(Naples)*
Soil-borne beneficial microbes that improve food security

Graziano Zocchi *(Milan)*
The long and winding road of iron from soil to seed

6. Population genomics

Chair: Giovanni Destro Bisol *(Sapienza, University of Rome)*

Mark Jobling *(Leicester, UK)*
Sex-specific histories from targeted resequencing of human and great ape genomes

Paolo Francalacci *(Sassari)*
Refining the human Y-chromosome tree using phylogenetically informative SNPs from the whole euchromatic region (23.1 Mbp)

Luca Pagani *(Cambridge, UK)*
Inferences on worldwide human diversity from a panel of 450 high coverage whole genome sequences

Enza Colonna *(Naples)*
Extreme pattern of genetic diversity in humans

11:00 - 11:30 Coffee break

11:30 - 12:30 Plenary Lecture

Johannes Krause *(Tuebingen, Germany)*
Genomic analysis of pre-contact Mycobacterium tuberculosis from the new world

Closing Remarks/Awards by Felice Cervone

PLENARY LECTURE

PL.1
Genome regulation by Polycomb proteins and 3D chromosome folding

Giacomo Cavalli, T. Sexton, C. Jacquier, P. Carrivain
Institut de Génétique Humaine, CNRS. 34396 Montpellier Cedex 5, France

Polycomb Group (PcG) and trithorax group (trxG) proteins form multimeric protein complexes that regulate chromatin via histone modifications and modulation of nucleosome remodeling activities, targeted to specific cis-regulatory elements named PcG response elements (PREs). However, they can also dynamically bind to other genes and affect cell proliferation and differentiation in a wide variety of biological processes. In particular, we are studying the role of different PcG genes as tumor suppressors during Drosophila development. Moreover, our ongoing research reveals a specific role for selected Polycomb proteins during oogenesis, and we are characterizing the molecular mechanisms underlying this function. In addition to silencing the genes flanking their genomic target sites, PcG proteins play a role in nuclear organization by forming large chromosomal domains and nuclear foci which are the physical sites of Polycomb-mediated silencing. Moreover, endogenous PcG target loci can frequently colocalize in the cell nucleus. These contacts depend on Polycomb proteins and stabilize epigenetic gene silencing. Thus, the three-dimensional organization of the genome contributes to epigenetic memory of gene expression states. Recently, we mapped genome-wide chromosome contacts in the fruit fly genome by ultra-deep Hi-C and described the domain organization of chromosomes as well as long-range contacts involving distant chromosome domains, either of the Polycomb type or of heterochromatin, null chromatin or active chromatin. We are now using mathematical modeling in order to develop algorithms that can predict probabilities of chromosome contacts in vivo. Here, I will discuss our progress in these subjects.

Acknowledgements: This work was supported by a European Research Council Advanced Investigator grant (FlyingPolycomb), by the Agence Nationale pour la Recherche (iPolycomb), by the Association pour la Recherche sur le Cancer, as well as by the CNRS.

PL.2
Huntington, the story of an ancient gene in search of a better future

Elena Cattaneo
Department of Biosciences, University of Milan

Huntingtin (htt) is the ~800 million-year old protein product of the Huntington's disease (HD) gene. The gene contains a polymorphic tri-nucleotide CAG repeat that is translated into polyglutamine amino acid (polyQ) residues in the protein. When this polyQ stretch at the 18 aminoacid (aa) position of the protein expands to over 36 residues, HD occurs, a fatal, genetically dominant, neurodegenerative disease. The CAG repeats are well conserved in deuterostomes, which suggests that they are an ancestral feature retained during the evolution of the protein. Htt carries a number of specific activities in the adult brain; for instance, it promotes transcription of neuronal genes among which is the BDNF, a neurotrophin critical for the survival and activity of cortical and striatal neurons that degenerate in HD. This presentation will highlight the power of combining evolutionary and developmental approaches to the study of the biology of disease-genes and will review the more recent discoveries of the function of htt in the developing and mature brain.

PL.3
The unfolded protein response in plants — facing a changing world

Stephen Howell
Plant Sciences Institute, Iowa State University, Ames IA 50014

Given the realities of global climate change, there is great concern about the ability of plants to tolerate environmental stress. A system in plants that perceives and responds to abiotic stresses, particularly stress brought about by elevated temperature, is the unfolded protein response (UPR). Protein folding is a environmentally sensitive process in plants and under adverse conditions, misfolded proteins accumulate in the endoplasmic reticulum, eliciting the UPR. The UPR activates stress sensor/transducers on the ER membrane which upregulate stress response genes in the nucleus. Plants have two types of stress sensor/transducers – one type involving membrane-associated transcription factors, such as bZIP8, that are mobilized in response to stress and relocate to the nucleus. The other involves the RNA splicing factor IRE1, which in response to stress splices a mRNA encoding another transcription factor, bZIP60, that also upregulates stress response genes. The consequence of activating the ER stress sensor/transducers is to elicit autophagy and other processes to mitigate stress damage under mild stress conditions or programmed cell death under more severe conditions. The analysis of mutants in Arabidopsis, demonstrates that the UPR protects both vegetative and reproductive development in plants against stress. Male gametophyte and pollen development are particularly vulnerable to temperature stress and the UPR plays a critical role in protecting these processes from stress.

PL.4
Genomic analysis of pre-contact *Mycobacterium tuberculosis* from the New World

Johannes Krause[1], K.I. Bos[1], A. Herbig[1], K.M. Harkins[2], J.E. Buikstra[2], S. Gagneux[3], A.C. Stone[2]
[1]Department of Archaeological Sciences, University of Tuebingen, [2]School of Human Evolution and Social Change, Arizona State University, [3]Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute

Using high throughput sequencing in combination with DNA capture techniques we have recently shown a phenomenal molecular preservation for medieval *Mycobacterium leprae* DNA from European skeletons, suggesting that genomic-level analyses of related pathogens such as *Mycobacterium tuberculosis* from similar aged skeletons would be feasible. Modern genomic analyses of M. tuberculosis suggest a worldwide distribution of this infamous pathogen following human dispersals out of Africa around 60,000 years ago. Here we report three approximately 1000-year-old ancient mycobacterial genomes from human remains found in Peru, confirming the presence of a member of the MTB complex in the pre-contact New World. The ancient strains are however distinct from any known human-adapted *M. tuberculosis* form and are most closely related to animal strains adapted to sea mammals. This is the first zoonotic transfer identified for *M. tuberculosis* with genetic evidence suggesting a re-adaptation to the human host and implicating a important role of sea mammals disseminating the disease across the ocean. Two independent dating analyses suggest a most recent common ancestor of all *M. tuberculosis* around 4,500 years ago implying a Holocene dispersal of the pathogen.

PLENARY SYMPOSIUM

PS1 - Microorganisms and Cancer

PS1.1
Carcinogenic properties of the bacterial genotoxins

R. Guidi[1], L. Del Bel Belluz[1], L. Levi[1], T. Frej Krejsgaard[3], S. Fazle[2], S. Puiac[2], M.G. Masucci[1], M. Rhen[2], **Teresa Frisan**[1]
Departments of [1]Cell and Molecular Biology, [2]Microbiology Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, [3]Department of International Health, Immunology and Microbiology, Copenhagen University, Denmark

Chronic inflammation and infection are associated with an increased risk of cancer development, however the mechanisms by which bacteria contribute to carcinogenesis are still poorly characterized. Several Gram-negative human pathogens produce the cytolethal distending toxin (CDT), which induces DNA damage in the target cells. The effects of intoxication are similar to those evoked by ionizing radiation, a well-characterized genotoxic stress, known to be carcinogenic. We are focusing on how CDT intoxication alters processes involved in the regulation of genomic integrity, cell cycle progression, cell survival as well as cytoskeleton dynamics and tissue remodelling *in vitro* and *in vivo*. We demonstrated that chronic exposure to sub-lethal doses of CDT in mammalian cells promoted the acquisition of malignant properties, such as genomic instability in the absence of significant alterations of cell cycle distribution, apoptosis or senescence. This phenotype was associated with impaired activation of the DNA damage response. Cell survival in response to DNA damaging agents is required for tumor initiation /progression, and we showed that cells exposed to the active CDT depend on sustained activity of the p38 MAP kinase pathway as well as on DNA damage-dependent integrin-mediated survival signals. To dissect the role of the CDT in *in vivo* tumor development, we produced a *Salmonella typhimurium* strain expressing *S. typhi* CDT-like toxin, known as typhoid toxin (TT), and as control, we used an isogenic strain carrying a mutant inactive. Both strains successfully infected the immunocompetent sv129 mouse strain for more than 2 months. We are currently analyzing the infection burden, the histopathology, the survival and the profile of the immune response in chronically infected animals. We are also characterizing the capacity of TT to modulate the host gut microbiota, since intestinal bacteria are known to play a crucial role in body homeostasis, inflammation and cancer onset in humans. Our results indicate that chronic exposure to CDT promotes the characteristic traits of tumor initiation/progression, alters the normal DNA damage responses and promotes cell survival, contributing to unravel the molecular mechanism(s) of bacterial-induced carcinogenesis.

PS1.2
Helicobacter pylori infection and Th17 profile in chronic gastritis

F. Munari*, M. Fassan†, G. Codolo*, M. Vila-Caballer*, M. Pizzi‡, M. Rugge‡, C. Della Bella§, S. D'Elios§, M.M. D'Elios§, **Marina de Bernard***
**Department of Biology, University of Padua, Padua, Italy, †ARC-NET Research Centre, University of Verona, Verona, Italy, ‡Department of Medicine – DIMED, Surgical Pathology & Cytopathology Unit, University of Padua, Padua, Italy, §Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy*

B cell activating factor, BAFF, is a crucial cytokine affecting the activity of both innate and adaptive immune cells. It promotes the expansion of Th17 cells in autoimmune disorders. With the present study, we investigated the BAFF/Th17 responses in Helicobacter pylori-induced gastritis in human. Our results show that the mucosa from Helicobacter-positive patients with chronic gastritis is enriched in IL-17 and BAFF whereas the two cytokines are weakly expressed in Helicobacter-negative patients with chronic gastritis; moreover, the expression of both BAFF and IL-17 decreases following bacteria eradication. We demonstrate that BAFF accumulates in macrophages in vivo and that it is produced by monocyte-derived macrophages (MDMs) in vitro, following Helicobacter stimulation. Application of BAFF on monocytes triggers the accumulation of ROS that are crucial for the release of pro-Th17 cytokines, such as IL-23, IL-1β and TGF-β. Moreover, BAFF directly promotes the differentiation of Th17 cells. In conclusion, our results support the notion that an axis BAFF/Th17 exists in chronic gastritis of Helicobacter-positive patients and that its presence strictly depends on the bacterium. Moreover, we demonstrated that BAFF is able to drive Th17 responses both indirectly, by creating a pro-th17 cytokine milieu through the involvement of innate immune cells, and directly via the differentiation of T cells towards the specific profile. The results obtained in this study are of great interest for Helicobacter-related diseases and the development of novel therapeutic strategies based on the inhibition of the BAFF/IL-17 response.

PS1.3
Role of human papillomaviruses in carcinogenesis

Massimo Tommasino
Infections and Cancer Biology Group, International Agency for Research on Cancer, Lyon, France

Infectious agents represent a major group of risk factors for cancer development and contribute to about 20% of human cancers worldwide. Seven viruses, i.e. human papillomavirus (HPV), hepatitis C virus (HCV), hepatitis B virus, Human T-lymphotropic virus type I (HTLV-1), Epstein-Barr virus (EBV), Kaposi sarcoma-associated virus (KSHV), Merkel Cell polyomavirus (MCPyV) and one bacterium, *Helicobacter pylori*, have been clearly associated with human carcinogenesis. HPVs are a family of small double-stranded DNA viruses that have a tropism for the epithelia of the genital and upper respiratory tracts and for the skin. Approximately 150 HPV types have been discovered so far, which are classified into several genera based on their DNA sequence. Approximately 15 high-risk mucosal HPV types are clearly associated with cervical cancer; HPV16 and HPV18 are the most carcinogenic since they are responsible for approximately 50% and 20% of all cervical cancers worldwide, respectively. It is now also clear that these viruses are linked to a subset of other genital cancers, as well as head and neck cancers. In addition, ongoing studies concerning a sub-group of HPV types that infect the skin suggest their involvement, together with ultraviolet radiation, in the development of non-melanoma skin cancer (NMSC). The strategies of our group is to perform functional studies to characterize the biological properties of specific infectious agents using in vitro and in vivo model systems, with the final aim to predict their possible role in human carcinogenesis. The rationale of our functional studies is based on the fact that viruses directly associated with human cancers have developed several mechanisms to efficiently evade the immune surveillance and to promote cellular transformation. In the last few years we have identified and characterized several oncogenic viral mechanisms involved in the evasion of the immune response and/or in cellular transformation. A few examples will be presented.

PS1.4
Molecular mechanisms of HBV-associated hepatocarcinogenesis

Massimo Levrero
"Sapienza", University of Rome, Italy

[No Abstract Received]

PS2 - Modeling cell pathophysiology by integrative network-based association studies

PS2.1
Interrogation of tumor-specific regulatory models elucidates actionable non-oncogene dependencies for the treatment of aggressive and chemoresistant tumors

Andrea Califano
Columbia University, New York, NY, USA

Completion of large-scale genomic sequencing and transcriptomic profiling effort has produced an unprecedented quantity of data for the study of human disease, including cancer. Yet, the much-heralded vision of using these data to improve diagnosis and treatment of human disease is still largely unrealized. For instance, other than a handful of previously established dependencies, such as those resulting from EGFR, ERBB2, ALK, MET, and BRAF mutations, treatment of hundreds of cell lines, representing multiple tumors and immortalized normal tissue with hundreds of small molecules has largely failed to highlight striking new associations between genetic alterations and chemosensitivity. Similarly, following the success of oncogene addition in CML, and in molecularly distinct subsets of breast and lung cancer patients, pharmacological targeting of oncogenes has shown relatively modest improvements, such as a 5-month life extension in melanoma patients with activating BRAF mutations. Indeed, most of the successful targeted agents in cancer, such as HDAC, PARP, PD-1, proteasome and glucocorticoid receptor inhibitors, target a much broader space of non-oncogene dependencies, resulting from complex, statistically undecipherable combinatorial patterns of genetic/epigenetic alterations, as well as autocrine and tumor-host signals. To address this emerging challenge we introduce the use of context specific regulatory networks as computational models for the systematic identification of actionable, non-oncogene tumor dependencies and of master regulators of chemoresistance. These models have been successful in the elucidation of master regulator proteins that are both necessary and sufficient to implement physiologic cell state transitions, as well as tumor initiation, progression, and chemosensitivity. For instance, among many other examples, analysis of tumor specific regulatory networks has revealed C/EBPβ, C/EBPδ and STAT3 as synergistic master regulators of the mesenchymal subtype of high-grade glioma, associated with worst prognosis in patients [1], TLX1, TLX3, and RUNX1 as a necessary regulatory module in T-ALL [2], AKT1 as a master regulator of glucocorticoid resistance in T-ALL [3], and a set of 13 competitive endogenous RNAs, such that deletion of any subset of them abrogates PTEN expression in glioma patients [4]. Here, we will discuss a novel cross-specie regulatory network analysis resulting in the elucidation of FOXM1 and CENPF as synergistic master regulators of malignant prostate cancer progression [5], whose inhibition using combination therapy abrogates tumorigenesis *in vivo*. Specifically, the role of these two proteins as an obligated, synergistic regulatory bottleneck in aggressive prostate cancer establishes them as both biomarkers and actionable therapeutic targets for the treatment of these tumors. As has been the case for other disciplines, such as chemistry and physics, we envision that introduction of computational, genome-wide models representing the physical processes that determine cell behavior has the potential to transform biology into a quantitative, model-based, predictive science.

PS2.2
The logic network underlying the anti-cancer activity of metformin: a systems approach

F. Sacco[1,2], P.F. Gherardini[1], S. Paoluzi[1], D. Posca[1], A. Silvestri[1], L. Castagnoli[1], M. Mann[2], **Gianni Cesareni**[1]
[1]Department of Biology, University of Rome Tor Vergata, Rome Italy, [2]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany

Metformin is the most frequently prescribed drug for type 2 diabetes patients. Aside from its clinically defined "insulin sensitizer" role, metformin is increasingly being described as a pleiotropic drug and activities as diverse as promotion of neurogenesis or adjuvant in anti tumor treatment are reported. Metformin perturbs the cell energy balance and depending on the cell context it rewires the signaling networks and promotes different responses. We have developed a novel strategy, based on the combination of high content multi-parametric analysis with logic modeling to map cell perturbations on complex logic networks. Such approach enabled us to recapitulate known anti-proliferative molecular mechanisms of metformin and additionally identify new pathways, including Hippo signaling and CDK2 activation, whose modulation is implicated in cell cycle control. This rewiring of the signaling network does not lead to cell death, per se, but instead makes cancer cells less sensitive to growth stimuli and more sensitive to apoptotic drugs. The strategy that we have developed here is generally applicable to large-scale proteomic studies that aim at identifying physiologically relevant changes after cell perturbation in a variety of biological contexts.

PS2.3
Networking drugs and diseases: a systems biology perspective

Diego Di Bernardo
Tigem, Naples, Italy

Network-based drug discovery aims at harnessing the power of graph theory and visualisation to investigate the mechanism of action of existing drugs, or new molecules, in order to identify innovative therapeutic treatments. We will present our recent work in the field of network pharmacology, starting with approaches relying on computational models of transcriptional networks, then moving to "drug networks". We will show how large scale gene expression profiles consisting of hundreds of thousands experiments can be "summed up" in a network to reveal unanticipated connections between drugs and diseases. Molecular and drug networks are powerful integrated computational and experimental approaches that will likely speed up and improve the drug discovery process, once fully integrated into the academic and industrial drug discovery pipeline.

PS2.4
Cracking the naive Pluripotency code

S.-J. Dunn[1], **Graziano Martello**[2,4], B. Yordanov[1], S. Emmott[1], A.G. Smith[2,3]
[1]Computational Science Laboratory, Microsoft Research, Cambridge, UK, [2]Wellcome Trust, Medical Research Council Cambridge Stem Cell Institute, Univ. of Cambridge, Cambridge, UK, [3]Dept of Biochemistry, Univ. of Cambridge, Cambridge, UK, [4]Dept of Molecular Medicine, Univ. of Padua, Padua, Italy

Embryonic stem (ES) cells are from the pre-implantation mouse embryo; they can differentiate into all cell types of the adult organism, an ability defined as Naive pluripotency. External inputs, such as the LIF and Wnt signals, control the behavour of ES cells through activation of downstream transcription factors (Martello et al, 2012 and2013). Still, the gene regulatory circuitry through which pluripotent embryonic stem (ES) cells choose between self-renewal and differentiation appears vast and has yet to be distilled into an executive molecular program. Here we developed a data-constrained, computational approach to reduce complexity and derive a set of functionally validated components and interaction combinations sufficient to explain known ES cell behavior. Furthermore, our computational model predicts unknown and non-intuitive responses to compound genetic perturbations with an overall accuracy over 70%. We propose that propagation of ES cells can be explained by a relatively simple process of molecular computation (Dunn and Martello, *Science* 2014). It will be instructive to use our model to study how this state is generated in the developing embryo or by reprogramming.

PS2.5
Genomic Integration Sites (IS) analysis of virus-based vectors for gene therapy: the *Integrome*

Ermanno Rizzi[1], A. Moiani[2], M. Severgnini[1], F. Mavilio[2], G. DeBellis[1]
[1]Institute for Biomedical Technologies (ITB), National Research Council (CNR), Segrate (MI), 20090, Italy, [2]Genethon, 1bis Rue de l'Internationale, 91020 Evry, France

Virus-based vectors are used in gene therapy to replace mutated gene, but due to their unspecificity, the activation of oncogenes might occur, resulting in unexpected diseases, such as leukemia. The analysis of genomic integration sites (IS) of virus-based vectors is necessary to understand the virus insertion mechanisms and to identify vectors with a limited genotoxic activity. The collection of all IS, the *Integrome*, was explored using Ligation Mediated-PCR (LM-PCR) coupled to Next Generation Sequencing (NGS). LM-PCR allowed the amplification and enrichment of vector-genome junctions while NGS provided a high-coverage characterization of ISs. This combined strategy was applied on the *Integromes* of different retroviral and lentiviral vectors such as MLV (Moloney Murine Leukemia Virus), ASLV (Avian Sarcoma-Leukosis Virus) and HIV (Human Immunodeficiency Virus) in $CD34^{+}$human hematopoietic stem/progenitor cells. *Integrome* sequencing allowed us to better understand the insertion strategies of considered vectors, comparing their different integration patterns. The *Integrome* analysis will help the research to identify safer vectors as candidate for *ex vivo*gene therapy applications.

PS2.6
Role of the chromatin remodelling factor ISWI intTissue regeneration

G. D'Angelo[1], **Davide F.V. Corona**[1]
[1]Dept STEBICEF, University of Palermo, Palermo, Italy

During tissue regeneration cells that undergo fast rounds of proliferation can reprogram their epigenetic memory in order to regenerate parts of missing tissue. Recent studies suggest that chromatin factors have a critical role in the reprogramming of epigenetic cell memory events occuring during tissue regeneration. ISWI, a nucleosome stimulated remodeling factor conserved in all metazoans, is the ATPase subunit of multiple chromatin remodeling complexes known to have an important role in various biological processes. Using a combination of in vivo and in vitro approaches in the model system *D. melanogaster*, we found that ISWI places an essential role in the regulation of chromatin remodelling events occurring during tissue regeneration.

PS3 - Metabolic changes in tumor cells

PS3.1
MicroRNAs as master regulators of the biology of the tumor microenvironment

Muller Fabbri
University of Southern California Children's Hospital, Los Angeles, USA

After more than a decade of intensive research in the field, microRNAs (miRNAs) have established themselves as key players in human carcinogenesis. A plethora of published papers shows their dys-regulation in almost all types of human malignancies, and identifies them as excellent cancer biomarkers with diagnostic, prognostic and predictive-of-response-to-therapy implications. Recent evidence has shown that miRNAs can also be functionally transferred from one cell to another inside of microvesicles called exosomes. This previously unknown paracrine miRNA-mediated inter-cellular cross-talk mechanism has highlighted a new function for miRNAs within the tumor microenvironment. This lecture will focus on these novel mechanisms of action of miRNAs (which represent "heresies" from the traditional functions of the miRNome) and reveal a surprisingly new aspect of cancer biology, providing novel strategies to develop anti-cancer therapies.

PS3.2
Role of the inhibitor protein (IF_1) of the mitochondrial ATP synthase on tumor cells metabolism

Alessandra Baracca, G. Sgarbi, S. Barbato, G. Gorini, G. Solaini
Department of Biomedical and Neuromotor Sciences, University of Bologna, Via Irnerio 48, 40126 Bologna, Italy

Mitochondria employ the transmembrane proton motive force (pmf) to drive the mechanical rotary mechanism that leads to phosphorylation of ADP to ATP. When pH and mitochondrial membrane potential ($\Delta\psi m$) decrease, as in ischemia, the ATP synthase works in reverse hydrolyzing cytosolic ATP to maintain the transmembrane electrochemical gradient. This process is controlled by the inhibitor protein IF1 that binds to the F1 catalytic domain, to avoid the wasting of glycolytic ATP (Solaini G and Harris DA, 2005). Interestingly, a number of papers report that IF1 is over-expressed in various human cancer cells (Sánchez-Cenizo L et al., 2010; Solaini G et al., 2011), and recent evidence suggest a direct involvement of the inhibitor protein in triggering a ROS-mediated prosurvival and proliferative response in a variety of cell types, but the underlying molecular mechanisms remain poorly understood. In order to explore the relationship between the IF1 expression level and the bioenergetics of tumour cell lines, we here compare tumour cells with cell clones transfected with an IF1-shRNA plasmid, that generated permanently stable IF1 knockdown clones (IF1-KD cells).This model can help to clarify the ambiguities caused by transient and heterogeneous cellular models (i.d. different and variable expression levels of IF1) and possibly to identify molecular targets for the development of new therapeutic approaches.

PS3.3
Between life and death: a dual function for the mitochondrial F-ATP synthase

Paolo Bernardi
Department of Biomedical Sciences, University of Padova, Italy

The "permeability transition" (PT) denotes a Ca^{2+}-dependent permeability increase of the inner mitochondrial membrane to solutes with molecular masses up to about 1,500 Da. Recognized since the early days of mitochondrial research, its nature remained a mystery for 60 years. The modern era of PT research followed the demonstration that permeabilization (which is mediated by a proteinaceous pore, the PTP) is inhibited by cyclosporin A through its interaction with matrix cyclophilin (CyP) D; and that PTP opening may play a key role in apoptotic and necrotic cell death. We recently discovered that dimers of the mammalian F-ATP synthase can form Ca^{2+}- and oxidant-activated channels that possess all the electrophysiological features of the PTP. I will discuss recent data on channel formation by yeast and Drosophila F-ATP synthases suggesting that this novel feature of the enzyme may be a conserved evolutionary feature.

PS3.4
OXPHOS and PKM2 within tumor-stroma interplay: a new druggable synergy

Paola Chiarugi
Department of Experimental and Clinical Biomedical Sciences, University of Florence, Viale Morgagni 50, 50134 Florence, ITALY

The ability of cancer cells to invade and metastasize is influenced by the surrounding tumor microenvironment. It is established that cancer associated fibroblasts (CAFs) promote tumor progression by enhancing cancer cell invasiveness and stemness. In addition the reciprocal interaction between CAFs and prostate cancer (PCa) cells has been demonstrated to induce their metabolic reprogramming. Notably, both tumor microenvironment and metabolic reprogramming have been included in the revised model of the Hallmarks of Cancer. Interestingly, upon tumor-stroma interaction, CAFs undergo Warburg metabolism (i.e. increased glucose consumption and lactate extrusion), while PCa cells undergo a "reverse Warburg metabolism". This metabolic switch allows PCa cells to reactivate OXPHOS and exploit CAF-derived lactate to drive anabolic pathways, thereby supporting cell growth. We observed that the metabolic reprogramming of PCa cells is strictly dependent on a CAF-mediated inactivation of the M2 isoform of the pyruvate kinase (PK-M2), an enzyme largely expressed by cancer cells. In particular, we observed that CAFs contact induces in PCa cells (i) PK-M2 phosphorylation mediated by Src tyrosine kinase and (ii) PK-M2 oxidation mediated by the CAF-induced pro-oxidant environment. These events lead to PK-M2 inactivation, granting for its nuclear migration and association with hypoxia-inducible factor (HIF-1). The complex PK-M2/HIF-1 is responsible for the recruitment of the transcriptional repressor Differentially Expressed in Chondrocytes-1 (DEC-1), which in turn promotes the down-regulation of miR-205, a mandatory event for the execution of the epithelial-mesenchymal transition (EMT) program. Treatment of PCa cells with DASA-58 (a chemical activator of PK-M2) or Metformin (an inhibitor of mitochondrial respiratory chain complex I) interferes with PK-M2 nuclear translocation and association with HIF-1/DEC-1, ultimately abrogating the EMT and the pro-invasive spur in PCa cells, as well as their "reverse Warburg metabolism". Our data suggest an intriguing role for PK-M2 in coupling the motile and the metabolic programs, proposing a direct connection between the EMT program and the metabolic switch. PK-M2 pharmacological targeting could thereby allow to simultaneously impair both motile and metabolic features of cancer cells, with clear therapeutic advantages.

PS3.5
CSCs hypothesis claims for a re-evaluation of cancer metabolism

Roberto Scatena
Università Cattolica, Rome, Italy

Cancer stem cells (CSCs) or tumor-maintaining cells or cancer stem-like cells are a subpopulation of cancer cells that show some of the characteristics of stem cells to survive and adapt to ever-changing environments. These include the ability to self-renew and the capacity to produce progenitors that differentiate into other cell types. Some molecular aspects related to CSCs can significantly modify the experimental and clinical approaches to cancer and are becoming a key area of cancer biology . In fact, CSCs represent the ideal justification for a lot of intriguing and obscure aspects of cancer pathogenesis (i.e., cancer cell dormancy, chemoresistance, local and distant relapses). Considering the enormity of the clinical implications related to CSCs, a careful identification of the molecular phenotype associated to an accurate

definition of their typical plasticity which appears intriguingly related to derangements in cell differentiation and metabolism, can represent a fundamental advance in terms of early diagnosis and selective therapy of cancer. At last but not least, the knowledge of pathogenetic mechanisms at the basis of CSCs can enlarge and ameliorate the therapeutic applications of the normal adult stem cells (i.e., regenerative medicine, tissue engineering, biotechnology applications) by reducing the risk of a deranged, uncontrolled, and thereby potentially tumorigenic stem cell differentiation.

PARALLEL SYMPOSIA

S1 - The multiple facets of genome instability

S1.1
Causes and effects of karyotype alterations

Daniela Cimini
Virginia Tech University, Blacksburg, VA, USA

Cancer cells display distinct aneuploid karyotypes and typically mis-segregate chromosomes at high rates, a phenotype referred to as chromosomal instability. While it is readily apparent how chromosome mis-segregation can cause aneuploidy, the effect of aneuploidy on chromosome segregation is unclear. We recently investigated this problem by using primary cells, as well as immortalized and cancer cell lines carrying specific trisomies. We found that trisomy 7 or 13, but not 12, are associated with high rates of chromosome mis-segregation that affect certain chromosomes more than others. Further, we found that overexpression of the cytokinesis regulator *SPG20* (Spartin) on chromosome 13q13.3 caused cytokinesis failure in both cancer and primary cells with trisomy 13. Overall, these data show that aneuploidy can, *per se*, induce chromosome mis-segregation and that different aneuploidies can yield distinct cellular phenotypes. Moreover, we investigated the effect of tetraploidy on karyotype stability and found that tetraploidy promotes karyotypic heterogeneity not because of increased chromosome mis-segregation rates, but because of increased tolerance for chromosome numerical imbalances. Overall, our data indicate that changes in chromosome number promote karyotipic heterogeneity. This, in turn, may promote phenotypic changes and phenotypic heterogeneity, thus conferring adaptability to aneuploid cancer cell populations.

S1.2
Mutant cohesin drives chromosomal instability in early colorectal adenomas

F. Cucco[1,2], A. Servadio[3], V. Gatti[4], P. Bianchi[5], L. Mannini[1], A. Prodosmo[4], E. De Vitis[1], G. Basso[5], A. Friuli[1], L. Laghi[5], S. Soddu[4], G. Fontanini[3], **Antonio Musio**[1,6]
[1]Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Pisa, Italy, [2]Dipartimento di Biologia, Università degli Studi di Pisa, Pisa, Italy, [3]Dipartimento di Patologia Chirurgica, Medica, Molecolare e di Area Critica, Università di Pisa, Pisa, Italy, [4]Oncologia Sperimentale, Istituto Nazionale Tumori Regina Elena, Roma, Italy, [5]Istituto Clinico Humanitas, Rozzano (Mi), Italy, [6]Istituto Toscano Tumori, Firenze, Italy

Chromosome missegregation leads to chromosomal instability, thought to play a role in cancer development. As cohesin functions in guaranteeing correct chromosome segregation, increasing data suggests its involvement in tumorigenesis. In a screen of a large series of early colorectal adenomas, a precocious step during colorectal tumorigenesis, we identified eleven mutations in *SMC1A* core cohesin subunit**.** In addition, we sequenced the *SMC1A* gene in colorectal carcinomas and we found only one mutation. Finally, the transfection of the *SMC1A* mutations identified in early adenomas and wild-type *SMC1A* gene silencing in normal human fibroblasts led to chromosomal instability. Our findings that *SMC1A* mutations decrease from early adenomas to colorectal cancers and that mutations lead to chromosomal instability suggest that mutant cohesin could play a pivotal role during colorectal cancer development.

S1.3
The dynamics of cancer genomes during disease progression

Francesca Demichelis
CIBIO, University of Trento, Italy

Cancer genomes are characterized by a variety of aberrations that accumulate over time and contribute to tumor progression. Recent studies highlighted the manifestation of clustered multi-rearrangement events (chromothripsis) and of abundant deletions and translocations that originated in an interdependent way across multiple chromosomes (chromoplexy). The characterization of all such events and of their clonal status through computational means is key in depicting tumor progression patterns and in understanding the clonal origin of tumor metastases. In an attempt to understand if a single clone metastasizes in patients affected by prostate cancer and remains dominant over the course of lethal disease we applied novel analysis strategies to deep-targeted sequencing data from serial plasma and tumor samples. We identified multiple independent clones in metastatic disease that are differentially represented in tissue and circulation and showed a temporal association between clinical progression and emergence of mutations. Resistant clones showed a complex dynamic with temporal and spatial heterogeneity suggesting distinct mechanisms of resistance at different sites that emerged and regressed dependent on treatment selection pressure. It is becoming evident that the clonal architectural heterogeneity at different stages of disease progression and clone dynamics can be used to understand the biological mechanisms behind disease progression but also to eventually inform patients management.

S1.4
Triplet repeat instability and human disorders

Stéphanie Tomé
Inserm UMR 1163 Laboratory of CTG repeat instability and myotonic dystrophy – Sorbonne Paris Cité, University, Imagine Institute, PARIS

Among repetitive DNA sequences, expansions of trinucleotide repeats (TNRs) were identified as a dynamic mutation in more than 40 neurological and neuromuscular disorders, such as Huntington's disease (HD). Above a size threshold, TNRs become pathogenic and highly unstable in tissues (somatic instability) and across generations (intergenerational instability). Instability is expansion-biased, size- and age-dependant and contributes to variability in age-of-onset and progression of symptoms. The levels of somatic mosaicism are extremely variable between individuals and the source of this variability remains unknown. A greater understanding of this variability will improve clinical diagnosis and prognosis of TNR diseases, and will contribute to the development of new therapeutic strategies aiming to modulate repeat dynamics. We have addressed this question in a transgenic HD mouse model that faithfully recreates expansion-biased, age-dependent somatic mosaicism. We have shown that nucleotide changes in the DNA repair gene *Msh3* modulate protein levels and modify the degree triplet repeat somatic mosaicism, where lower protein expression are associated with reduced repeat instability. Our findings reveal that naturally occurring variations in DNA repair genes in humans may explain the variability of TNR instability and may serve as a predictor of disease onset and progression in patients.

S2 - Epigenetics: disturbance or resource for plant improvement?

S2.1
The epigenetic guardians of genome stability in plants

Marie Mirouze
IRD, Montpellier, France

In the last decade, plant biologists and plant breeders have developed a growing interest for the study of epigenetics, defined as the study of heritable changes in gene expression that cannot be explained by changes in the DNA sequence. Epigenetic regulations are involved in plant development, environmental response and control of genome stability: their study thus represents a great promise to better understand plant adaptation to changing environmental conditions. In plants, transposable elements (TEs) play a major role in shaping the epigenome. Our current project proposes to have an insight on the consequences of crop epigenetic misregulation on TE activity and on plant phenotype. In this project we will analyze the repertoire of mobile TEs or 'mobilome' in Arabidopsis and in rice, the impact of active TEs on host phenotypes either through genetic mutation or through epigenetic changes (creation of epialleles), and the role of the host in restricting TE movement in response to stress conditions. I will present the preliminary results obtained in characterizing different plant mobilomes and will discuss the possible transfer of this new technique to different crops.

S2.2
Histone acetylation and sex-specific recombination variation in *Arabidopsis thaliana*

Pasquale Termolino
CNR-IBBR, Portici, Italy

Arabidopsis is characterized by heterochiasmy: indeed, it is reported that crossover (CO) rate and distribution vary between male and female meiosis. Chromosomes recombine at a higher rate in male than female meiosis (CO per cell: 11.1 *vs* 6.6). Furthermore, CO rates are high at the telomeric ends of the chromosomes in male meiosis and at proximal regions in female meiosis. Our research team investigated whether histone acetylation was determinant in heterochiasmy. To address this question, we analyzed chromosome-wide recombination frequency through SNP genotyping in populations derived from a T-DNA mutant *(Atmcc1*) characterized by increase in histone acetylation. Firstly, two F1 populations were generated by crossing *Atmcc1* as male and female parent with Ler genotype as well as two F1 control populations (reciprocal crosses C24 x Ler). Afterwards, four BC1 populations (1680 plants in all), obtained as following [F1(Ler x C24) x Ler], [Ler x F1(Ler x C24)], [F1(Ler x *Atmcc1*) x Ler] and [Ler x F1(Ler x *Atmcc1*)], were genotyped by 143 SNPs homogenously distributed on the whole genome with a mean interval length of 800 kb. Genotyping was performed using the patented KASP SNP genotyping system that uses Fluorescent Resonance Energy Transfer (FRET) probes on an arrayed 96 well plate resulting in high throughput and very efficient SNPs detection. Wide analysis revealed that histone acetylation is not determinant for heterochiasmy. Furthermore, genotyping of *Atmcc1* evidenced that T-DNA insertion was associated to a reciprocal translocation involving chromosomes 3 and 4. Interestingly, as a consequence of chromosome rearrangements a global redistribution of crossovers along chromosomes occurred.

S2.3
Epigenomics of stress response in maize leaf

Serena Varotto
University of Padua, Italy

A current challenge in population genetics is to demonstrate that epigenetic variants, stably inherited through generations and associated to phenotypic variation, exist both dependently and independently of cis- or trans-acting DNA sequence changes. In crops, this challenge can be address both using natural population sampled from a wide geographical area and mutation accumulation line developed in the labs, for instance applying different types of stress of agronomic interest. To address the role of stress-induced epigenetic gene and TE regulation in maize, we analyzed the effects of salt and drought stresses on whole genome transcriptional modulation both in B73 inbred line and the epiregulator mutant *rmr6*, a PolIV mutant impaired in short interfering RNA biogenesis and in the RNA-directed DNA methylation pathway. A total RNA-Seq strategy has been used to analyze the transcriptome of wild type and mutant leaves after ten days of stress application and after 7 day of recovery period. Illumina sequenced reads have been mapped on the maize reference genome, to analyze the expression of annotated genes and to create a new annotation of our "stress-specific transcriptome" including TEs and long-non-coding RNAs (lncRNAs). Gene expression analysis revealed the modulation of many stress-related genes and the transcription of thousands of novel loci. Many of these loci encode for non-annotated TEs and their characterization is underway. The transcriptomic data were also integrated at genome-wide level both with the small RNA dataset (sRNA-Seq) and the distribution of chromatin marks (ChIP-seq for H3K4me3, H3K9ac and H3K27me3 histone modifications) exploring the whole epigenomic landascape of stress response and adaptation in maize. ChIP-seq analysis of the three histone modifications are revealing that the stress treatment altered the epigenomic landscape and we are currently evaluating the entity of these changes in the leaf treated tissues.

S2.4
Challenged from inside and outside: plant epigenetic responses to genomic modifications and environmental cues

Emanuele De Paoli
University of Udine

Epigenetic modifications refer to heritable changes in gene expression and phenotype caused by mechanisms other than alterations in the primary DNA sequence. Cytosine methylation is one of the best characterized mechanisms and in plants it occurs at level of gene promoters and transposable elements where it strongly correlates with transcriptional repression and silencing. The same epigenetic modification is also present in the transcribed regions of active genes suggesting, in this case, a positive role in gene expression. Multiple observations reinforce the view that DNA methylation is pivotal in shaping the functional landscape of eukaryotic genomes including crop plants. Conversely, genomic structural modifications like those induced by transposable elements as well as environmental cues may trigger plant responses with effects on genomic methylation patterns and gene regulation. Indeed, what is most intriguing about DNA methylation changes as a result of internal (genomic) or external (environmental) challenges is their potential inheritance, which highlights the relevance of plant epigenetic responses in determining the genetic features of next generation plants.

S2.5
Use of MSAP markers to analyze the effects of abiotic stresses on DNA methylation

G. Marconi, M. Bocchini, F. Veronesi, **Emidio Albertini**
Dept of Agricultural, Food and Environmental Sciences, University of Perugia, Italy

DNA methylation is a heritable epigenetic enzymatic modification resulting from the addition of a methyl group in the cyclic carbon-5 of cytosine. In higher plants, heavy cytosine methylation has been found to play an important role in gene expression as promoter regions of silent genes have been found to be more methylated than actively transcribed sequences. Moreover, DNA methylation in plants is generally species-, tissue-, organelle- and age-specific. In fact, changes in DNA methylation are present throughout the entire life cycle of plants, starting from seed germination up to the plant death either programmed or induced by various agents such as biotic and abiotic stresses. Among several methods for detecting DNA methylation and the methylation-sensitive

amplified polymorphism (MSAP) technique was developed to asses the extent and pattern of cytosine methylation in the genomes of several species. The technique is based on the use of the isoschizomers HpaII and MspI, that differ in their sensitivity to methylation of their recognition sequences. We have applied the MSAP technique to several species subjected to abiotic stresses (drought, salt, iron, etc) with the aim to identify epigenetic changes due to stresses and to investigate the role of methylated gene-sequences in the adaptation to these stresses. Experimental data will be presented and discussed.

S3 - Biochemistry of intracellular channels, carriers and pores

S3.1
Introduction: Distinct biochemical features and functional roles of VDAC isoforms of outer mitochondrial membrane

Vito De Pinto
Department of Biological, Geological and Environmental Sciences, Section of Molecular Biology, University of Catania, Italy and National Institute for Biomembranes and Biosystems, Section of Catania, viale A. Doria 6, 95125 Catania, Italy

This Symposium aims to address a field that is now emerging as crucial for unravelling the intracellular mechanisms of recognition and regulation of the metabolic flow: the usually small proteins that, with various mechanisms, are the conduit for ions or molecules across the intracellular membranes defining the subcompartments and organelles. These proteins, (carriers, channels and pores), once difficult to be isolated and characterized due to their inherent paucity, are now becoming an affordable subject and are investigated with the most advanced and ultrasensitive molecular techniques. In this symposium, thus, there are some of the most advanced and productive groups in this field, to will testify the high level of the italian research and its potentiality in basic and applied research projects (1-4). It is noteworthy that most information about the membrane proteins allowing the chemical communication between the sides of the hydrophobic barrier comes from mitochondria: the power plant but also the regulatory hub for functions as apoptosis, autophagy and cellular plasticity. The outer mitochondrial membrane (OMM) is not essential for the production of ATP: however, it is the doorway, the external envelope that controls the access and possibly regulates the flow of energy from/to the organelle. The most abundant group of proteins in the OMM are the mitochondrial porins termed VDAC (Voltage Dependent Anion selective Chanels). In our recent work we are trying to elucidate the role of the most elusive of the components of the family: the isoform VDAC3. We have characterized the functional properties of this pore that has a much smaller conductance than the other isoforms (5). The proteins interacting with VDAC3 have been studied with a sensitive interactomic technique in living cell (6). The results obtained point to a role of this isoform not overlapping the simple picture of a wide, unspecific pore but suggesting a more intricate function as a connector at the surface of mitochondria. In this respect studies about VDACs are revealing that the various isoforms should have differentiated roles (7).

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S3.2
Role of VDAC in Regulating Mitochondrial Respiration in Cancer Cells and in Neurodegenerative Disease

Tatiana Rostovtseva
Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

The crucial role of mitochondria in energy production, calcium signaling, promoting apoptotic signals, and neurodegeneration is well-established. A central question is how mitochondria control cell survival and cell death. We address this question by studying the voltage-dependent anion channel (VDAC), the conduit for ATP and other mitochondrial metabolites fluxes across the mitochondrial outer membrane (MOM). We have found that dimeric tubulin, the subunit of microtubule, induces highly efficient reversible blockage of VDAC reconstituted into planar lipid membranes. Although the tubulin-blocked state is still conductive for small ions, it is virtually impermeable for ATP. We propose that by blocking VDAC permeability for ATP and other respiratory substrates, tubulin controls mitochondrial respiration. These findings are supported by experiments with isolated mitochondria and human hepatoma cells, thus uncovering a previously unknown mechanism of regulation of mitochondrial energetics by free tubulin and also suggesting why and how cancer cells preferentially use inefficient glycolysis rather than oxidative phosphorylation (the Warburg effect).Direct implication of small intrinsically disordered neuronal protein alpha-synuclein (α-syn) in Parkinson disease (PD) has been well documented. However, the exact mechanism of its toxicity and effect on neuronal mitochondria in particular remain elusive. Recently we have shown the existence of a functional interaction between VDAC and α-syn. We found that at nanomolar concentrations α-syn reversibly blocks reconstituted VDAC channel. The analysis of α-syn-VDAC binding kinetics suggests that α-syn not only blocks but translocates through VDAC and, therefore, could target complexes of the mitochondrial respiratory chain in the inner membrane. These findings explain the previously reported effects of α-syn on mitochondrial dysfunction suggesting a new mechanism of regulation of mitochondrial function by α-syn. To support our *in vitro* findings, we explored a yeast model of PD and showed that α-syn toxicity in yeast depends on VDAC. These results suggest that interaction of α-syn with VDAC could be essential for physiological adaptation of mitochondrial respiration and dysfunction in neurodegenerative disease. Taken together, our data point out to that VDAC regulation mediated by its association with various cytosolic proteins could be implicated in a wide variety of mitochondria-associated pathologies.

S3.3
Viral K^+ channels teach us lessons on protein sorting

T. Guthmann[1], C. von Chappuis[1], A. Moroni[2], **Gerhard Thiel**[1]
[1]From Membrane Biophysics, TU-Darmstadt, Schnittspahnstrasse 3, 64287 Darmstadt, Germany, [2]Department of Biology and CNR IBF-Mi, and Istituto Nazionale di Fisica della Materia, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy.

Our seminal observation was that two very similar viral encoded K^+ channels are sorted to different cellular locations: one channel, Kcv, is trafficking via the secretory pathway to the plasma membrane whereas a second channel, Kesv, is targeted to the inner membrane of mitochondria. We now use these two proteins to examine the mechanism, which is responsible for this non-canonical intracellular protein sorting. In previous experiments we found that insertion of ≥2 hydrophobic amino acids near the end of the c-terminal transmembrane domain (TMD2) in Kesv was sufficient for redirecting the sorting of this channel; the Kesv mutants were no longer sorted to the mitochondria but to the plasma membrane. To detect the structural information in TMD2, which decides the sorting pathway, we use a randomization approach. For this purpose we employ a yeast complementation assay to identify mutants that target

Kesv to the plasma membrane after randomization of the amino acid sequence in TMD2. The data confirm the contribution of TMD2 in Kesv for sorting. But a detailed analysis of the mutants shows that neither the length nor the hydrophobicity of TMD2 is entirely responsible for the differential sorting of the small Kesv channel protein.

S3.4
Inhibition of a mitochondrial potassium channel as a strategy against pancreatic ductal adenocarcinoma

A. Managò[1], A. Zaccagnino[2], L. Leanza[1], B. Linder[2], H. Kalthoff[2], **Ildiko Szabo**[1]
[1]Department of Biology, University of Padova, Italy, [2]Institut für Experimentelle Tumorforschung, University of Kiel, Germany

Mitochondrial ion channels are emerging as oncological targets. Among the potassium channels found in the inner mitochondrial membrane we identified mtKv1.3 as a possible target. By preventing the function of this channel using specific membrane permeant inhibitors (Psora-4, PAP-1 and clofazimine), cancer cells of different origin expressing Kv1.3 undergo cell death. In particular, inhibition of the channel causes increased mitochondrial ROS production and cytochrome c release. The cells become resistant to these Kv1.3 modulators when expression of the channel is downregulated by siRNA. We have previously shown that in vivo treatment of melanoma in an orthotopic mouse model with clofazimine caused 90% reduction of the tumor volume without affecting healthy tissues. Furthermore, these inhibitors efficiently killed primary human tumor Chronic lymphocytic leukemia (B-CLL) cells expressing Kv1.3 by intrinsic apoptosis, while sparing residual normal T-lymphocytes even of the same patient and B cells from healthy subjects. Since Kv1.3 inhibitors kill B-CLL by direct interference with mitochondrial functions, they act on these malignant cells independent of classic prognostic factors, Bcl-2 overexpression and Bax/Bak deficiency. Here we report the expression profile of Kv1.3 in five different pancreatic ductal adenocarcinoma lines under different conditions and the effect of Kv1.3 inhibitors and of their improved derivatives *in vitro*. In the case of some of these drugs, even staurosporine-resistant lines died upon treatment. All cell lines analyzed displayed mutation in p5321 and these cell lines have been tested previously to be largely resistant to standard chemotherapeutics. Clofazimine has been tested also *in vivo* in a SCID mouse model for PDAC, characterized by a local recurrent tumor and liver metastases closely resembling the clinical challenge.

S3.5
The biochemical function of uncoupling protein 2, unknown until now

A. Vozza[1], G. Parisi[1], F. De Leonardis[1], F. M. Lasorsa[1], A. Castegna[1], D. Amorese[1], L. Palmieri[1], D. Ricquier[2], E. Paradies[1], P. Scarcia[1], F. Palmieri[1], F. Bouillaud[2], **Giuseppe Fiermonte**[1]
[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, 70125 Bari, Italy, [2]Institut Cochin, INSERM-U1016 CNRS UMR9078, Université Paris Descartes, 75014 Paris France

Uncoupling protein-2 (UCP2) is involved in various cellular physiopathological processes such as the glucose and glutamine oxidation, diabetes, stem cell differentiation, ROS production and cancer. Attempts to explain the numerous metabolic alterations associated with gain or loss of function of UCP2 have focused on potential roles in uncoupling mitochondrial respiration from ATP generation through a less efficient UCP1-like protonophoric activity. Although this «uncoupling theory» has been questioned no alternative mechanisms have been demonstrated up to date. Being UCP2 a member of the mitochondrial carrier family, we tested the hypothesis that transport of substrates, other than protons, could better explain its metabolic effects. The bacterially-expressed human UCP2 reconstituted into liposomes was shown to exchange aspartate, malate and oxaloacetate with phosphate. Yeast phosphate carrier-knockout mitochondria expressing UCP2 catalyzed an uptake of Pi and H^+. The higher levels of citric acid cycle intermediates in the mitochondria of siUCP2-HepG2 compared to those of wild-type cells and the transport data indicate that UCP2 catalyzes an exchange of intramitochondrial four carbon (C4) intermediates for cytosolic Pi by a H^+-assisted mechanism. By exporting C4 out of mitochondria UCP2 regulates the entry of acetyl-CoA in the Krebs cycle and the mitochondrial energetic potential. Our work reveals a novel regulatory mechanism in cell bioenergetics and provokes a substantial reconsideration of the physiological and pathological functions ascribed to UCP2 based on its purported uncoupling properties.

S3.6
Cation transporters in health and disease: novel functions and role as drug targets

L. Pochini, M. Galluccio, M. Scalise, **Cesare Indiveri**
Laboratory of Biochemistry and Molecular Biotechnology, Department BEST (Biologia, Ecologia, Scienze della Terra), University of Calabria, Italy

The three members of the Organic Cation Transporter Novel subfamily evolved after the divergence of vertebrates and invertebrates. The transporters are characterized by large hydrophilic loops which challenge 3D structure resolution. Therefore, only bi-dimensional structural models are available, highlighting 12 transmembrane segments. OCTN2 (SLC22A5) is the best characterized member of the subfamily. It catalyzes Na^+-dependent carnitine transport and is responsible of carnitine homeostasis, essential for life. OCTN1 (SLC22A4) is still poorly characterized. It was proposed to transport generic cations or the fungi metabolite ergothioneine. Recently acetylcholine has been identified as the physiological substrate. Acetylcholine transport is involved in non-neuronal cholinergic functions controlling inflammation. Indeed OCTN1 mutation is associated with Inflammatory Bowel Diseases. OCTN3 (SLC22A21) is involved in carnitine accumulation in mouse testis. Surprisingly, octn3 is missing in the human genome and the player of carnitine accumulation in human testis is obscure. The OCTN transporters interact with xenobiotics and drugs, thus being of primary interest in human health.

S4 - Telomeres in model organisms: similarities and differences with human telomeres

S4.1
Protection of telomeres: mechanisms and regulation in *S. cerevisiae*

Maria Pia Longhese
Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy

The natural chromosome ends need to be distinguished from intrachromosomal DNA double-strand breaks, which are targeted by DNA repair pathways and activate the major DNA damage-induced checkpoint kinases ATM and ATR. Protection of chromosome ends from degradation and unwanted double strand break repair is achieved by a highly ordered nucleoprotein complex called telomere. The telomeric structure and most of the proteins that bind double- and single-stranded telomeric DNA are conserved from yeast to mammals, suggesting that the mechanisms underlying telomere identity are based on common principles. I will present the mechanisms that govern the proper terminal structure of *Saccharomyces cerevisiae* telomeres, as well as the pathogenetic consequences that arise when this structure is modified.

S4.2
Organization and Evolution of *Drosophila* Telomeres

Giovanni Cenci[1,2], G. D. Raffa[1], L. Ciapponi[1], M. Gatti[1,3]
[1]Dipartimento di Biologia e Biotecnologie "C. Darwin," Sapienza Università di Roma, Roma, Italy, [2]Istituto Pasteur-Fondazione Cenci Bolognetti, Sapienza Università di Roma, Roma, Italy [3]Istituto di Biologia e Patologia Molecolari del CNR, Sapienza Università di Roma, Roma, Italy

Drosophila lacks telomerase and fly telomeres are elongated by occasional transposition of three specialized retroelements. *Drosophila* telomeres do not terminate with GC-rich repeats and are assembled independently of the sequence of chromosome ends. Recent work has shown that *Drosophila* telomeres are capped by the terminin complex, which includes the fast-evolving proteins HOAP, HipHop, Moi, and Ver. These proteins, which are not conserved outside *Drosophilidae* and closely related Diptera, localize and function exclusively at telomeres, protecting them from fusion events. Other proteins required to cap telomeres in flies include HP1, the E2 ubiquitin-conjugating enzyme Eff/UbcD1, ATM, the components of the Mre11-Rad50-Nbs (MRN) complex, and the Woc transcription factor. These proteins do not share the terminin properties; they are evolutionarily conserved non-fast-evolving proteins that do not accumulate only at telomeres and do not serve telomere-specific functions. We propose that upon loss of telomerase, *Drosophila* rapidly evolved terminin to bind chromosome ends in a sequence-independent manner. This suggests that terminin is the functional analog of the shelterin complex that protects human telomeres. The non-terminin proteins are instead likely to correspond to ancestral telomere-associated proteins that did not evolve as rapidly as terminin because of the functional constraints imposed by their involvement in diverse cellular processes. Thus, the main difference between *Drosophila* and human telomeres is likely to rely on the protective complexes that specifically associate with the DNA termini. We believe that the study of *Drosophila* telomeres provides excellent opportunities for investigations on human telomere biology. The identification of additional *Drosophila* genes encoding non-terminin proteins involved in telomere protection might disclose novel components of human telomeres.

S4.3
Zebrafish telomerase is required for normal lifespan and cancer

Miguel Godinho Ferreira
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Telomere dysfunction in vertebrates has been primarily studied in inbred mice strains with very long telomeres that fail to deplete telomeric repeats during their lifetime. It is, therefore, unclear whether telomere shortening regulates tissue homeostasis in vertebrates with naturally short telomeres. Zebrafish have restricted telomerase expression and human-like telomere length. We have recently shown that, in contrast to lab mouse models, first generation tert-/- zebrafish die prematurely with shorter telomeres. tert-/- fish develop degenerative phenotypes, including premature infertility, gastrointestinal atrophy and sarcopaenia. Thus, telomerase is limiting for zebrafish lifespan, enabling the study of telomere shortening in naturally aging individuals. Contrary to other cancers, metastatic melanoma remains practically incurable and is responsible for 90% of skin cancer deaths. BRAFV600E tp53- /- zebrafish develop melanoma ranging from invasive to metastatic. Our studies show that absence of telomerase prevents the onset of melanoma and thus required for all stages of carcinogenesis and metastasis. We are currently investigating whether telomerase loss leads to complete melanoma eradication or, alternatively, severely delays the onset of tumorigenesis.

S5 - Plant-soil relationships as key factors for food security and sustainability

S5.1
Omics approaches to unravel plant-soil relationships

Paolo Nannipieri
Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università di Firenze

The rhizosphere soil is the site of complex interactions between plant and soil biological, chemical and physical properties, with the former, particularly those due to microbial activities, being more sensitive in response to rhizodepositions. Plant roots can have detrimental, beneficial and neutral interactions with soil microbial communities inhabiting rhizosphere soil. Usually interactions between plant and beneficial microorganisms (for example, with plant growth promoting rhizobacteria) occur through molecular cross talks. Sometime the interaction also involves fauna as it is the case of the so called "microbial loop" in the plant N nutrition. The use of various omics techniques (mainly metagenomics, metatranscriptomics, proteomics and protogenomics) hold much promise in better understanding the plant soil microbial communities interactions but also await further refinement before they are ready for widespread adaptation. One way to judge their readiness is to compare them to methods that have become standards for soil microbiology research. Methods become standards because they provide useful information quickly and inexpensively. Research has been mainly devoted to gene detection rather than gene expression. There is no question that omics can provide useful information, some of which cannot be obtained with traditional techniques, and integration of omics methods may provide insights into ecosystem functioning. In particular, the potential for omics to provide comprehensive coverage of genes and genes products make them well-suited for the study of general soil microbiological phenomena, such as decomposition, response to water stress, etc. Nannipieri P, Pietramellara G, Renella G (2013) Omics in Soil Science. Caster Academic Press, Norfolk, UK

S5.2
Soil metagenomics. Potential and pitfalls

Pascal Simonet
Environmental Microbial Genomics group, Ampère-UMR CNRS 5005, ECL and University of Lyon, 69134 Ecully cedex, France

Bacteria cultivability imitation can be circumvented by modern alternative approaches including metagenomics or single cell genomics. Metagenomics includes the data treatment of DNA sequences from many members of the microbial community, in order to either extract a specific microorganism's genome sequence or to evaluate the community function based on the relative quantities of different gene families. In my talk I will show how these metagenomic datasets can be used to estimate and compare the functional potential of microbial communities from various environments with a special focus on antibiotic resistance genes. Metagenomics can also be used to exploit the genetic potential of environmental microorganisms. I will present an integrative approach coupling *rrs* phylochip and high throughput shotgun sequencing to investigate the shift in bacterial community structure and functions after incubation with chitin. In a second step, these functions of potential industrial interest can be discovered by using hybridization of soil metagenomic DNA clones spotted on high density membranes by a mix of oligonucleotide probes designed to target genes encoding for these enzymes. After affiliation of the positive hybridizing spots to the corresponding clones in the metagenomic library the inserts are sequenced, DNA assembled and annotated leading to identify new coding DNA sequences related to genes of interest with a good coverage but a low similarity against closest hits in the databases confirming novelty of the detected and cloned genes.

S5.3
Soil-borne beneficial microbes that improve food security

Matteo Lorito[1,2], M. Ruocco[2], F. Vinale[2], R. Marra[1], S. Lanzuise[1], N. Lombardi[1], R. Varlese[1], G. Manganiello[1], A. Pascale[1], S. Woo[1,2]
[1]Department of Agriculture, University of Naples, and [2]Institute for Plant Protection of CNR, Via Università 100, 80055 Portici (Naples) e.mail: lorito@unina.it.

Finally, the new EU directive 2009/128 is going to promote widely the use of biological control agents as active ingredients of biopesticide or biofertilizer formulations, either as living microbes, extracts or bioactive molecules. It will be possible to place the really useful products supported by a solid scientific base on a fast track to gain a much larger market share. In fact, the required move from chemical to IPM-based disease management will correspond to the loss of an estimated 50% of the synthetic active principles available today, and has to be to be fully implemented within the next 24 months. Soil-borne beneficial microbes are gathering an increasing attention of scientists and industry due to their ability to provide a variety of benefits to the plant and generally increase quality and safety of the agriculturally-produced food. In addition, they have been found to secrete a variety of secondary metabolites that may be particularly useful for applications in agriculture. The presentation will summarize the actual state of the art, and show how the introduction of new biological or naturally-derived products in the market is currently driven by 'omics-based strategies for the selection and combination of the active principles.

S5.4
The long and winding road of iron from soil to seed

Graziano Zocchi
DISAA - Universita degli studi di Milano

Iron (Fe) is an essential element for all living organisms, since it is a cofactor of proteins and enzymes involved in a wide range of biochemical functions in the plant energy management such as respiration and photosynthesis. In humans Fe deficiency is probably the major nutritional constraint affecting almost 2.7 billion people, in particular children and young women in developing countries. To ameliorate this deficiency several approaches have been made. One of the most promising is the biofortification of plants (grains and vegetables) to increase the amount and the bioavailability of iron in their edible parts. The task is quite complex since it is important to understand how plant take up this microelement from the soil and above all how it will be delivered to the edible parts of the plant. In this talk I will present and discuss the possible routes iron goes through from soil to the seeds and how is stored in more bioavailable forms.

S6 - Population genomics

S6.1
Sex-specific histories from targeted resequencing of human and great ape genomes

Mark A. Jobling
University of Leicester, Leicester, UK

Recent advances in technology allow us to sequence whole human genomes, but this is still prohibitively expensive at the population level. Sequence data are usually at low coverage, leading to poor ascertainment of rare or unique variants, and difficulties with interpreting paralogous sequences. An alternative approach is to target specific sub-regions of the genome, and sequence these at high coverage. We have used this approach to sequence segments of the sex chromosomes and autosomes in 448 human and 22 great ape males, totalling ~26 Mb each. Human samples include 17 European populations, allowing us to investigate European prehistory from a multi-locus perspective. Analysis of 4.4 Mb of Y-chromosomal DNA defines an unbiased phylogenetic tree containing 13,261 high-confidence single-nucleotide polymorphisms (SNPs) in total, and 5996 among European samples. Dating of the major clades indicates that 64% of the sampled chromosomes descend from only three ancestors who each lived 3.0-6.0 KYA. In demographic reconstructions a continuous swathe of 13/17 populations from the Balkans to the British Isles share similar histories featuring a reduction in effective population size ~2-3 KYA, followed by expansion to the present. Together with data on maternally inherited mitochondrial DNA, this demonstrates a recent widespread male-specific phenomenon that may indicate social selection, and refocuses interest on the social and population structure of Bronze Age Europe. Analysis of autosomal and X-linked sequences is ongoing, and includes a systematic examination of gene conversion in the evolution of the sex chromosomes. Cross-species sequence capture is efficient, and yields high-coverage data for ape orthologs thus providing outgroups for human data, and also illuminating sex-specific differences among species.

S6.2
Refining the human Y-chromosome tree using phylogenetically informative SNPs from the whole euchromatic region (23.1 Mbp)

Paolo Francalacci
University of Sassari, Italy

Previous studies on genetic variation within the male specific portion of the Y chromosome (MSY) based on next-generation sequencing were limited to the study of the X-degenerate regions. From low coverage whole genome sequencing of 1,194 Sardinian males, we extracted 20,155 phylogenetically informative single nucleotide polymorphisms (SNPs) from the whole MSY euchromatic region, including X-degenerate, X-transposed, Ampliconic and "Other" classes, and from the readable sequences of the heterochromatic region. The variants were used to construct a phylogenetic tree at high resolution that contained all main haplogroups found in Europe — highlighting the high degree of inter-individual differentiation of Sardinians — along with many new lineage clusters within each haplogroup. The non X-degenerate classes contain a significant portion of the phylogenetic variation of the Y chromosome and their inclusion in the analysis refines the known phylogeny, confirms previous estimates about the age of the Most Recent Common Ancestor, and provides a deeper insight into the history of the peopling of Sardinia.

S6.3
Inferences on worldwide human diversity from a panel of 450 high coverage whole genome sequences.

Luca Pagani[1]
on behalf of the International Consortium of the Estonian Centre for Genomics, [1]Division of Biological Anthropology, University of Cambridge, UK

Complete high coverage individual genome sequences carry the maximum amount of information for reconstructing the evolutionary past of a species in the interplay between random genetic drift and natural selection. Here we present a novel dataset of 450 human genomes sequenced at 40X on the same platform (Complete Genomics), processed on uniform bioinformatic pipelines. Based on SNP-chip data we chose on average three samples to represent each of the 156 populations of interest. The spatial coverage achieved by the sampling strategy implemented here retrieved high resolution, continent-wide patterns of heterozygosity, archaic admixture and changes in effective population size over time. Furthermore the estimation of pairwise genetic split times between single genomes using MSMC (Shiffels et al. 2014) enabled us to refine the current understanding of peopling dynamics leading from Africa into Eurasia, Oceania and America highlighting potential multiple waves of arrival in Papua New Guinea.

S6.4
Extreme pattern of genetic diversity in humans

Enza Colonna
CNR, Naples

The understanding of genetic variation is essential to decode traces that evolution has left in our genomes and whole-genome sequence data now allow us to do interpret these signals at a resolution never possible before. Genetic variation in humans generally follows clines defined by geographical regions, and there are possibly very few fixed differences between any pair of continents or populations. Nevertheless, genetic differences among populations exist, reflecting mainly past demographic events and in certain cases they can be quite extreme. Both patterns of too much or too little differences exists in different regions of the same human genome and I will illustrate those cases. First, I will talk about ultrasensitive and transcribed ultraconserved regions where there is very little genetic differentiation as a consequence of negative selection. Secondly, I will show the case of region that present extreme differences between populations (highly differentiated sites) as adaptation to population-specific environmental pressure, or positive selection. Finally, I will talk about cases of population-specific lack of genetic variability due to consanguinity and isolation. Elucidating the functional causes of these extreme patterns of population differentiation remains the major challenge.

POSTER AND ORAL PRESENTATIONS

1 - Metabolism and its regulation in health and diseases

P1.1
Hepcidin binding to ferroportin prevents exocrine pancreatic failure and fatal iron overload

S. Altamura[1], R. Kessler[1], H.J. Groene[2], N. Gretz[3], B. Galy[4,5], M.W. Hentze[4,5], M.U. Muckenthaler[1,5]
[1]Department of Pediatric Hematology, Oncology and Immunology - University of Heidelberg, Germany, [2]Deutsches Krebsforschungszentrum Heidelberg, Germany, [3]Medical Faculty Mannheim, Germany, [4]European Molecular Biology Laboratory, Heidelberg, Germany, [5]Equal contribution

Systemic iron levels are tightly controlled by the hepatic hormone hepcidin in response to iron availability, inflammation or hypoxia. Hepcidin binds to the iron exporter ferroportin (FPN) to regulate iron release from exporting cells. A FPN mutation (C326S) was reported in a patient with hereditary hemochromatosis (HH) type 4 and shown to abrogate hepcidin binding in vitro. To study consequences of the disruption of the hepcidin−ferroportin interaction in vivo, we generated the first mouse model of HH type 4. At 8−weeks of age FPNC326S knock-in mice show profoundly increased transferrin saturation and serum ferritin levels as well as hepatic iron overload. Macroscopically, C326S mice show progressive brown discoloration of the pancreas that correlates with profound iron deposition, increased reactive oxygen species and degeneration of exocrine pancreatic cells. Starting at the age of 33 weeks, pancreatic failure is accompanied by progressive wasting and death. We believe that C326S FPN mice represent the first example of fatal iron overload in an animal model, opening avenues to investigate the underlying molecular mechanisms.

P1.2
Platelet-activating factor acetylhydrolase, paraoxonase 1 and oxidized low density lipoproteins in Alzheimer's disease patients and elderly subjects

T. Bacchetti[1], A. Vignini[2], L. Nanetti[2], A. Giulietti[2], L. Mazzanti[2], G. Ferretti[2]
[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, [2]Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche, Università Politecnica delle Marche

Paraoxonase-1 (PON1) and platelet activating factor acetylhydrolase (PAF-AH) are multitasking enzymes associated to lipoprotein surface. They play a key role in lipoprotein functions and it has been suggested that alterations of their activities are involved in disorders associated with inflammation and oxidative stress. Aim of our study was to investigate the activities of PON1 and PAH-AH in Alzheimer's disease (AD), a neurodegerative diseases associated with oxidative damage. PON1 paraoxonase and arylesterase activities and PAF-AH activity were evaluated in plasma from 49 patients affected by AD and from 34 control subjects matched for gender and age. Moreover, the levels of oxidized LDL (ox-LDL), a useful marker of lipoprotein peroxidation, were measured in plasma of the same subjects. Our results demonstrated alterations in plasma activities of PON1 and PAF-AH in AD patients with respect to controls and showed, for the first time, a relationship between the activities of these enzymes, ox-LDL levels and severity of the disease. In conclusion, PAF-AH and PON1 appear to be an important link between lipoprotein functions, oxidation and inflammation in Alzheimer's disease.

P1.3
Silencing of FLAD1 gene affects neuromuscular transmission in *Caenorhabditis elegans*

E. Dipalo[1], V. C. Liuzzi[2], T. A. Giancaspero[2], G. Zampi[3], E. Di Schiavi[3], C. De Giorgi[1], M. Barile[1,2]
[1]Dip. di Bioscienze, Biotecnologie e Biofarmaceutica,Università degli Studi di Bari "A. Moro", Bari, Italy, [2]Istituto di Biomembrane e Bioenergetica, CNR, Bari, Italy, [3]Istituto di Bioscienze e BioRisorse, CNR, Napoli, Italy

We have identified *C. elegans R53.1*, namely *FLAD1*, as the gene coding for FAD synthase, an ubiquitous enzyme catalysing the biosynthesis of the redox cofactor FAD, allowing for the biogenesis of many flavoenzymes involved in cell bioenergetics and regulation (Barile M et al, Curr Pharm Des 2013, 19:2649). The phenotype of *FLAD1*-silenced worms was characterized to be severely affected, with impairment in fertility, protein homeostasis and locomotion possibly due to altered cholinergic transmission (Liuzzi VC et al, BBA 2012, 1820:521). Here we further investigate the effect of *FLAD1* silencing on the expression of flavoenzymes of pivotal importance in energetic metabolism, demonstrating a significant reduction of the protein amount of ETF-QO, accompanied by a significant transcription increase. Moreover, while the visualization of motor neuron morphology is under investigation, we provide further evidence of the involvement of *FLAD1*in cholinergic transmission by comparing control and silenced worms with respect to egg laying behaviour, with/without serotonin treatment and locomotion behaviour after treatment with cholinergic agonists and inhibitors. In these aspect *FLAD1*-silenced worm could represent an animal model system for studying human neuromuscolar diseases associated with alteration in flavoenzyme biogenesis.,**This work was supported by PON 01_00937 grants to MB.*

P1.4
Modeling human coasy mutation in yeast *Saccharomyces cerevisiae*

C. Ceccatelli Berti[1], E. Tosi[1], S. Dusi[2], V. Tiranti[2], P. Goffrini[1]
[1]Department of Life Sciences, University of Parma, Italy, [2]Unit of Molecular Neurogenetics IRCCS Foundation Neurological Institute "C. Besta" Milano, Italy

Neurodegeneration with brain iron accumulation (NBIA) comprehends a wide spectrum of clinically and genetically heterogeneuos diseases characterized by neurodegeneration and iron overload in specific brain areas. Mutations in *PANK2*, encoding for the mitochondrial enzyme panthotenate kinase 2 which catalyzes the first step in the Coenzyme A (CoA) biosynthesis, account for about 50% of NBIA cases. Coenzyme A (CoA) is a key metabolite in all living organisms being involved in several metabolic processes including metabolism of fatty acids, carbohydrates, amino acids and ketone bodies. Recently Coenzyme A synthase (COASY) gene has been identified as a novel NBIA-associated gene, supporting the concept that a dysfunction in CoA synthesis may play a role in the pathogenesis of NBIA. To gain more insights into the potential role of CoA in NBIA and of its relationship with brain iron accumulation, we have developed a yeast model that expresses a pathogenic COASY mutation found in patients. In this model phenotypic and biochemical investigation such as measurement of the CoA levels, assessment of OXPHOS, sensitivity to oxidative stress has been performed.

P1.5
Serglycin knockdown reduces inflammatory response in articular murine chondrocytes stimulated with LPS

A. D'Ascola[1], M. Scuruchi[2], A. Avenoso[1], A. Calatroni, S. Campo[1], G.M. Campo[1]
[1]Dept Biomedical Sciences and Morphological and Functional Images, Univ. Messina, Italy, [2]Dept Clinical and Experimental Medicine, Univ Messina, Italy

Serglycin plays a role in inflammatory response by interacting with the

hyaluronan receptor CD44, but there are limited data on the functions of this PG in chondrocytes. In this study we investigated the effect of serglycin knockdown on normal mouse chondrocytes stimulated with lipopolysaccharide (LPS). LPS induced an increase in serglycin and CD44 mRNA and related protein levels. Stimulation of chondrocytes with LPS also produced NF-kB activation, with a consequent transcription of pro-inflammatory mediators, such as tumor necrosis factor-alpha (TNF-alpha), inteleuking-1beta (IL-1beta), inteleuking-18 (IL-18) and inducible nitric oxide synthase (iNOS). Serglycin inhibition by specific small interference RNA (siRNA) decreased CD44 levels and inflammatory response. The treatment of LPS-stimulated chondrocytes with specific synthetic HA blocking peptide (Pep-1) in part reduced CD44 and pro-inflammatory mediators up regulation, without being able to completely abolish the serglycin pro-inflammatory effect.These results suggest that serglycin is involved in the inflammatory response in chondrocytes stimulated with LPS, suggesting a possible role in cartilage inflammation.

P1.6
Might Cterm LeuRS peptide sequences be used to rescue defects due to mitochondrial tRNA mutations?

M. Fazzi D'Orsi, A. Montanari, L. Zhou, A. Presciutti, G. Carraco, L. Frontali, S. Francisci
Dept Biology and Biotechnologies "C.Darwin", Univ. "La Sapienza"

Mt diseases are multisystem disorders due to mutations in nuclear or mtDNA genes. Substitutions in tRNA genes are responsible for a wide range of pathologies. Today no treatments are available. We have previously shown that the overexpression of genes coding for mt LeuRS, mt ValRS or mt IleRS, all belonging to class I and subclass a, were able to suppress the pathological phenotypes associated with mutated mt tRNAs both in human and in yeast. In view of potential therapeutic applications, some relevant issues need to be addressed and we aim at establishing: the extent of cross suppression among different mt aaRS; whether the C-terminal regions of human mt IleRS and ValRS are endowed with suppressing and cross-suppressing abilities analogous to the Cterm region of LeuRS previously observed; whether short sequences (β30_31 and β32_33) derived from the Cterm domain of mt LeuRS are able to rescue the defective phenotypes due to mutations in different tRNAs. By band-shift experiments we observed that the β30_31 peptide interacts specifically with both WT and mutated human mttRNALeu and mttRNAIle. Sequences derived from the LeuRS Cterm should be preferentially investigated for therapy.

P1.7
Class I histone deacetylases: key players in the pathophysiology of metabolic disorders

A. Ferrari, E. Fiorino, S. Barilla, M. Giudici, N. Mitro, D. Caruso, U. Guerrini, E. De Fabiani, M. Crestani
Dip. di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano

Histone deacetylases (HDACs) are epigenetic regulators involved in metabolic homeostasis. We investigated the effect of class I HDAC inhibition in a model of obesity induced by a high fat diet. Class I selective HDAC inhibitor MS275 reduced body weight (10%) and improved glucose tolerance. MS275 treated mice could better counteract the reduction of body temperature during an acute cold exposure, as a result of the improved thermogenic capacity of brown adipose tissue (BAT) confirmed by higher expression of BAT markers (*Dio2*,*Elovl3*). In white adipose tissue (WAT) MS275 reduced adipocyte size and increased markers of fat functionality (*Glut4*, *Pparg*, *Fabp4*), lipid catabolism (*Cpt1b*, *Lcad*) and mitochondrial biogenesis (*Tfam*, *CytC*). Visceral WAT of MS275 treated mice showed increased oxidative capacity, as shown by increased expression (70%) of gene encoding for the uncoupling protein 1 *Ucp1*, suggesting "browning" of this fat depot. Our results demonstrated that class I HDAC inhibition stimulated functionality and oxidative potential of adipose tissue, inducing global insulin-sensitizing effects and ameliorating the metabolic profile in diet-induced obese mice.

P1.8
Regulation of NADPH cytochrome P450 oxidoreductase by 1,25-dihydroxyvitamin $D_3$and all-trans retinoic acid in acute myeloid leukemia cells

E. Gocek, A. Marchwicka, E. Marcinkowska
Faculty of Biotechnology, University of Wroclaw, Poland

Acute myeloid leukemia (AML) is a heterogenous group of malignant blood diseases affecting the myeloid lineage. Because of the variability of cytogenetic abnormalities contribute to AML phenotype, satisfactory treatment is not available. Differentiation-induced therapy seems to be promising alternative. AML cells can be driven to differentiate to monocyte-like cells by 1,25- dihydroxyvitamin D_3 (1,25D) and to granulocyte-like cells by all-trans-retinoic acid (ATRA). Both compounds activate their specific receptors, VDR and RARs respectively. Inside the cells 1,25D is degraded by CYP24, while ATRA is degraded by CYP26. NADPH cytochrome P450 oxidoreductase (POR) is a membrane-bound enzyme required for electron transfer to cytochrome P450 (CYP), vital in the processes of the metabolism of drugs and steroid production. Therefore we studied the expression of POR gene and protein in AML cells induced to differentiate by 1,25D and ATRA. It turned on that POR is upregulated by ATRA and by 1,25D at the level of mRNA and protein (in membranes and mitochondria). Partial silencing of POR in HL60 cells resulted in augmented differentiation in response to 1,25D.*Studies founded by the National Science Centre (0351/B/P01/2011/40), conference costs by the University of Wroclaw scholarship for the best PhD (BPZ.506.50.2012.MS)*

P1.9
Overexpression of superoxide dismutase 1 in yeast devoid of endogenous porin: effect on mitochondria and oxidative stress

A.Magrì[1], S. Reina[1], M. C. Di Rosa[1], M. F. Tomasello[2], A. Jaubert[1], V. De Pinto[1], A. Messina[1]
[1]Dept Biological, Geological and Environmental Sciences, Sect Biochemistry and Molecular Biology, University of Catania, Catania, Italy, [2]CNR – Institute of Biostructure and Bioimaging, Section of Catania, Italy

Human VDAC1, the most abundant porin of the outer mitochondrial membrane, is involved in metabolic cross-talk between mitochondria and cytosol [1]. It is highly conserved in eukaryotes: *S. cerevisiae* por1 shows 70% of sequence homology to hVDAC1 and similar functional properties. hVDAC1 is protected from carbonylation by the specific presence of superoxide dismutase 1 (SOD1), the most relevant enzymatic defense against ROS. Anyway, relationship between VDAC1 and SOD1 is more extensive. An yeast strains devoid of endogenous SOD1 show altered levels of VDAC activity associated to a lower expression of mitochondrial proteins (VDAC, Tom40) [2]. To unravel the role of SOD1 on VDAC-mediated metabolism we expressed hSOD1 in a yeast strain devoid of endogenous porin (Δpor1). While Δpor1 strain cannot grow in the presence of a not fermentable carbon source, possibly due to an altered mitochondria, our results indicates that the overexpression of hSOD1 in this yeast strain relieves the growth defect. We thus suggest that SOD1 is involved in the mitochondrial metabolic intersection with the cytosol.*[1] Messina et al, 2012, BBA 1818, 1466-1476, [2] Karakitos et al, 2009, FEBS Lett 583, 449–455*

P1.10
The pyruvate carrier, main mitochondrial substrate transporter. From early functional characterization to recent finding of pathological gene mutations.

S. Papa
IBBE, CNR at DSMBNOS University of Bari

The first evidence of the existence of a mitochondrial pyruvate carrier, MPC (Papa et al., FEBS Lett., 1971, 12, 285) and its functional characterization (Papa and Paradies, Eur. J. Biochem. 1974, 49, 265)

date back to the seventies. The carrier, since of its essential role for mitochondrial degradation to CO_2 of glycolytic pyruvate and the capacity of pyruvate/acetoacetate exchange- transport, plays a key role in aerobic energy metabolism, ketogenesis, fatty acid metabolism, etc. Depression of the pyruvate carrier was, in fact, found in mitochondria form different experimental tumors (Paradies et al., Cancer Research, 1983, 43, 5068). After nearly forty years, two independent groups (Bricker et al., Science, 2012, 337, 96; Herzig et al., Science, 2012, 337) identified a complex of two mitochondrial proteins, MPC1 and MPC2, and the genes of the pyruvate carrier. Mutations in the MPC1 started to be identified in different families with children affected by lactic acidosis (Bricker et al see above ref.). It will not be a surprise if further work will show deficiency of MPC in cases of human tumors, with high aerobic glycolysis, and in severe infantile lactic acidosis.

P1.11
Impaired Nrf2/ARE system contributes to SRB1 loss, as consequence of oxidative post-translational modifications, in *CDKL5* atypical Rett syndrome

A. Pecorelli[1,2], G. Belmonte[3], I. Meloni[4], G. Zollo[1,2], C. Sticozzi[5], F. Cervellati[5], C. Gardi[1], C. Signorini[1], L. Ciccoli[1], A. Renieri[4,6], J. Hayek[2], G. Valacchi[5]

[1]Dept. Molecular and Developmental Medicine, Siena Univ., Siena, Italy, [2]Child Neuropsychiatry Unit, University General Hospital, Azienda Ospedaliera Universitaria Senese, Siena, Italy, [3]Dept. Medicine, Surgery and Neurosciences, Siena Univ., Siena, Italy, [4]Dept. Molecular Biotechnology, Siena Univ., Siena, Italy, [5]Dept. Life Science and Biotechnologies, Ferrara Univ., Ferrara, Italy, [6]Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy

CDKL5 mutations were related to atypical variant of Rett syndrome (RTT, *MECP2* mutations). Evidence indicates cholesterol homeostasis perturbation and oxidative-mediated loss of HDL receptor SRB1 in RTT. Here, we analyze whether cholesterol homeostasis is perturbed also in *CDKL5*. Lipid serum profile of 16 *CDKL5* patients was determined. Fibroblasts from 4 *CDKL5* and 4 healthy subjects were examined for SRB1, redox status and antioxidant defence by immunoblotting and immunofluorescence. Higher serum cholesterol with a significant decrease of SRB1 protein but significant increase in mRNA was detected in *CDKL5* fibroblasts. An increase in 4-hydroxynonenal (HNE) and 3-nitrotyrosine (NT) protein adducts associated with a significant reduction of Nrf2 is also observed. Finally, HNE and NT oxidative-mediated post-translational modifications of Nrf2 and SRB1 were demonstrated. In summary, our findings suggest that the altered lipid metabolism is a possible common pathological mechanism for *MECP2* and *CDKL5* RTT. HNE- and NT-induced modifications of Nrf2 and SRB1 can cause impairment and loss of two proteins, contributing to perturbation of redox status and lipid homeostasis in *CDKL5*.

P1.12
Novel synthetic thyronamines as new powerful tools to explore the physiological function of TAAR1

M. Sabatini[1], G. Chiellini[1], S. Rapposelli[2], G. Nesi[2], M. Digiacomo[2], S. Frascarelli[1], S. Espinoza[3], R. Zucchi[1]

[1]Dept. of Pathology, University of Pisa, Pisa, Italy, [2]Dept. of Pharmacy, University of Pisa, Pisa, Italy, [3] Italian Institute of Technology ,Genova, Italy

Thyronamine and 3-iodothyronamine (T1AM) are endogenous agonists of trace amine associated receptors type1 (TAAR1). While these molecules have great potential for a wide variety of therapeutic applications, their rapid catabolism might limit their therapeutic impact. In the present project we synthesized a small panel of synthetic thyronamines (SG-1,2,7,8), where the introduction of selected key structural modifications is expected to improve the biopharmacological profile and pharmacokinetic properties of the parent compounds. Bioluminescence Resonance Energy Transfer (BRET) based assays, using HEK-293 cells transfected with mTAAR1 and the cAMP BRET biosensor, showed that all compounds are effective mTAAR1 agonists. In particular, compound SG-2, which shares a close similarity to T1AM, was found to be the most potent with EC_{50} =240nM. In addition, as previously observed with T1AM, in the isolated working rat heart SG-1 and SG-2 produced a dose-dependent reduction of cardiac output, with IC(50)=40 μM, and 20 μM, respectively. In conclusion our results suggest that SG compounds can be used as new tools to explore the physiological function of TAAR1.

P1.13
Folate metabolism and Alzheimer's Disease

P. Tannorella[1,2], A. Stoccoro[2], G. Tognoni[3], P. Bongioanni[4], B. Nacmias[5], G. Siciliano[3,6], S. Sorbi[5], F. Coppedè[2], L. Migliore[2]

[1]Department of Biology, University of Pisa, Pisa, Italy, [2]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy, [3]Unit of Neurology, Department of Neuroscience, Pisa University Hospital, Pisa, Italy, [4]Unit of Neurorehabilitation, Department of Neuroscience, Pisa University Hospital, Pisa, Italy, [5]Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Florence, Italy, [6]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, It

Impairments of folate metabolism leading to increased homocysteine (Hcy) levels might contribute to Alzheimer's disease (AD), and genetic polymorphisms of folate metabolic enzymes have been suspected to contribute to those impairments as well as to sporadic AD risk. We investigated the contribution of polymorphisms of genes participating in folate metabolism in 418 AD patients, 77 individuals affected by Mild Cognitive Impairment (MCI), and 325 matched controls, and searched for correlation between each of them and data on Hcy, folate and vitamin B12 levels. We observed significant increased frequencies of the *MTHFR* 677T allele in AD patients, of both the *MTHFR* 1298C and the *MTRR* 66G alleles in AD and in MCI individuals with respect to controls. Significantly increased plasma Hcy levels and decreased serum folate values were observed in AD subjects with respect to controls. Several interactions among the studied polymorphisms and biochemical data were observed. Overall, present results support a contribution for one-carbon metabolism to AD pathogenesis. The study was supported by the Italian Ministry of Health (GR-2009-1606229; FC Principal Investigator)

P1.14
miRNAs: a connection between fatty acid metabolism and cancerogenesis in prostate cells

A. Valentino[1], S. Crispi[1], S. Filosa[1], S. Margarucci[1], R. Calogero[3], F. Riccardo[3], U. Galderisi[1,2], G. Peluso[1]

[1]Institute of Bioscience and BioResources; CNR; Naples; Italy, [2] Second University of Naples, Italy, [3]Dept Molecular Biotechnology and Health Sciences Univ., Torino, Italy

Prostate cancer is characterized by low glycolysis and increased fatty acid oxidation as a dominant bioenergetic pathway. miRNAs are a large family of RNAs that act as gene expression regulators at the post-transcriptional level, controlling several crucial cellular processes, such as fatty acid oxidation. Over-expression of some miRNA down-regulates expression of proteins, (i.e., CPT1), important for lipid metabolism, thus, decreasing β-oxidation of fatty acids. To better investigate the molecular mechanisms linked to the modulation of FA metabolism in prostate cancer, we analysed microRNAs (miRNAs) expression profiles using next-generation sequencing, in normal (PNT2) and cancer prostate cell lines (PC3 and DU145). Sequences have been mapped with respect to the miRNA precursors (mirbase v19) using SHRIMP aligner. miRNA differential expression have been detected by deseq software. These studies expanded our understanding of miRNA in the biological regulation of lipid homeostasis, and suggested that some specific miRNA may have important effects that extend beyond its role as metabolism regulator, accounting for the different FA metabolism in tumor cells.

O1.1

Impaired increase of plasma abscisic acid in response to oral glucose load in type 2 diabetes and in gestational diabetes

S. Bruzzone[1], P. Ameri[2], E. Mannino[1], G. Sociali[1], G. Andraghetti[2], A. Salis[1], M. Ponta[2], L. Briatore[2], G.F. Adami[3], A. Ferraiolo[4], P.L. Venturini[4,5], D. Maggi[2], R. Cordera[2], G. Murialdo[2], E. Zocchi[1]

[1]Dept of Experimental Medicine, Section of Biochemistry and CEBR, Univ. of Genova, Genova, Italy, [2]Dept of Internal Medicine, Univ. of Genova, Genova, Italy, [3]Dept of surgical sciences and integrated diagnostic, Univ. of Genova, Genova, Italy, [4]IRCSS San Martino IST, Genova, Italy, [5]Dept of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Mother and Child Sciences, Univ. of Genova, Genova, Italy

The hormone abscisic acid (ABA) regulates glucose homeostasis in humans. In normal glucose tolerant (NGT) human subjects, plasma ABA (ABAp) increases after an oral glucose load. In this study, treatment-naive T2D subjects and pregnant women with gestational diabetes (GDM) underwent an oral glucose load for diagnostic purposes (pregnant women underwent the glucose load before and after childbirth). ABAp increase in response to glucose was abrogated in T2D patients compared to NGT controls. The same was observed in the women with GDM compared to pregnant NGT controls; after childbirth, however, fasting ABAp, ABAp response to glucose and glucose tolerance were restored to normal in the GDM subjects. We also compared fasting ABAp before and after bilio-pancreatic diversion (BPD) in obese, but not diabetic subjects and in obese T2D patients, in which BPD resulted in the resolution of diabetes. Compared to pre-BPD values, ABAp significantly increased 1 month after BPD in all subjects, in parallel with a reduction of fasting plasma glucose. These results indicate an impaired hyperglycemia-induced ABAp increase in T2D and suggest a beneficial effect of elevated ABAp on glycemic control.

O1.2

Fibroblasts transfer lipids and proteins to cancer cells through cargo vesicles supporting tumor growth

A. Santi[1], A. Caselli[1], F. Ranaldi[1], P. Paoli, C. Mugnaioni[1], E. Michelucci[2], P. Cirri[1]

[1]Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università degli Studi di Firenze, Italy, [2]Mass Spectrometry Center, Università degli Studi di Firenze, Italy

Fibroblasts are the most abundant cellular elements in connective tissue and with the fibrillar extracellular matrix, within they are embedded, form the structural scaffolding of organs. Fibroblasts are responsible for the synthesis and remodeling of extracellular matrix and participate to the homeostatic regulation of adjacent epithelia through the secretion of growth factors or cytokines. In some pathological conditions, such as inflammation or cancer, fibroblasts transdifferentiate into their activated form. Here we report a novel, constitutive, property of fibroblasts related to their nutrients supply function, exerted by microvesicles-mediated transfer of biomass, *i.e.* proteins and lipids, to neighboring cells. This fibroblasts function is enhanced after their activation to myofibroblasts within tumor microenvironment. The most compelling effect of the massive transfer of proteins and lipids is the enhancement of tumor cells proliferation due to the increasing in the rate of mass accumulation to the lower limit necessary for cell division.

O1.3

Histone deacetylases (HDACs) and cholesterol catabolism: effect of HDAC7 deletion on lipid and lipoprotein profile

E. Fiorino, A. Ferrari, C. Multineddu, F. Gilardi, N. Mitro, D. Norata, P. Uboldi, A. Catapano, D. Caruso, E. De Fabiani, M. Crestani

Dip. Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Italia

The expression of Cholesterol 7α-hydroxylase (CYP7A1), the major check-point of bile acid (BA) synthesis, is repressed by BA. We showed that BA induce HDAC7, 3, 1 and SMRT recruitment on *CYP7A1* promoter and that non-selective HDAC inhibitors (HDACi) increase *CYP7A1* expression *in vitro* and *in vivo* by preventing BA repression and reduce serum cholesterol in mice. Based on these findings, our aim was to define the role of specific HDACs and corepressors in *CYP7A1* regulation and on cholesterol metabolism. We tested class selective HDACi*in vitro*: class I selective HDACi MS275 prevented BA repressive effect on *CYP7A1*. By mean of adenovectors we showed that Hdac1, 7 and Smrt silencing significantly increased *Cyp7a1* transcription. To study HDAC7 role *in vivo* we generated a HDAC7 liver-specific KO mouse and put it on western diet: we observed body weight and LDL-cholesterol reduction, increased liver BA consistent with increased *Cyp7A1* expression, lower liver lipid accumulation and a different HDL-cholesterol profile compared to wild type mice. Our results show that specific HDACs affect *CYP7A1* transcription highlighting their role in cholesterol and lipid homeostasis regulation.

O1.4

A mitochondrial switch promotes tumor metastasis

P. E. Porporato[1], V. L. Payen[1], J. Pérez-Escuredo[1], C. J. De Saedeleer[1], P. Danhier[1,2], O. Feron[1], B. Gallez[2], P. Sonveaux[1]

[1]Institute of Experimental and Clinical Research (IREC), Pole of Pharmacology, [2]Louvain Drug Research Institute (LDRI), Laboratory of Magnetic Resonance Imaging (REMA), University of Louvain (UCL) Medical School, Brussels, Belgium

Cancer metastasis is of dismal prognosis. Although aerobic glycolysis could promote metastasis, here we identified a different switch primarily affecting mitochondria. It corresponds to an overload of the electron transport chain (ETC) with preserved mitochondrial functions but increased mitochondrial superoxide production. The switch provided a metastatic advantage phenocopied by partial ETC inhibition, another situation associated with enhanced superoxide production. Both involved protein tyrosine kinases Src and Pyk2 as downstream effectors. Thus, two different events, ETC overload and partial ETC inhibition, promote superoxide-dependent tumor cell migration, invasion, clonogenicity and metastasis. Consequently, specific scavenging of mitochondrial superoxide with mitoTEMPO blocked tumor cell migration and prevented spontaneous tumor metastasis in murine and human tumor models.
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2 - Cellular Stress, apoptosis and autophagy

P2.1
Combinations of cytotoxic and pro-autophagic compounds against melanoma cells

G. Allavena[1,2], B. Del Bello[1,2], P. Tini[1,3], C. Miracco[1,3], L. Pirtoli[1,3], E. Maellaro[1,2]
[1]Istituto Toscano Tumori (ITT), 50139 Firenze, Italy, [2]Department of Molecular and Developmental Medicine, University of Siena, 53100 Siena, Italy, [3]Department of Medicine, Surgery and Neuroscience, University of Siena, 53100 Siena, Italy

Standard treatments against melanoma brain metastasis (combinations of Temozolomide (TMZ) and Ionizing Radiation (IR)) have poor efficacy, with a median survival rate of four months. Here, in human melanoma cell lines, we investigate cell proliferation and cell death induced by these single or combined therapies, in presence of Trehalose (TRE), a potent autophagy inducer. Results show that TMZ early induces a mild, protective autophagic response, followed, in sensitive cells, by morphological changes resembling senescence, cell growth arrest and apoptosis. The addition of IR enhances these effects, mostly affecting short-term cell proliferation, but it does not sensitize resistant cells towards apoptosis. Instead, a marked stimulation of autophagy, as induced by TRE treatment, while not impairing TMZ-induced apoptosis, potentiates both short- and long-term inhibition of cell proliferation. Interestingly, in sensitive cells, this effect is also evident in absence of IR. Therefore, possible combinations of Temozolomide (leading to mild autophagy and cell death) and Trehalose (leading to marked autophagy and cytostasis) are worth to be explored to overcome melanoma resistance.

P2.2
Changes in *SAM2*expression affect lactic acid tolerance and lactic acid production in *Saccharomyces cerevisiae*

N. M. Berterame, L. Dato, D. Porro, P. Branduardi
Department of Biotechnology and Biosciences, Univ. of Milano-Bicocca, Italy

The great interest in the production of highly pure lactic acid enantiomers comes from the application of polylactic acid (PLA) for the production of biodegradable plastics. Yeasts can be considered as alternative cell factories to lactic acid bacteria for lactic acid production, despite not being natural producers, whereas they can better tolerate acidic environments. Since high metabolite concentration represents the major limiting step of the process, our goal was the identification of novel targets possibly involved in the yeast response to lactic acid stress. Stress response implies the triggering of a complex physiological rearrangement, which is possible to obtain by modifying elements that have large effects on metabolic networks. The enzyme *S*-Adenosylmethionine (SAM) synthetase catalyses the only known reaction leading to the biosynthesis of SAM, a metabolite that participates in more reactions than any other cofactor with the exception of ATP. Here we will discuss how the modulation of *SAM*2 can have different outcomes upon lactic acid stress in different genetic backgrounds, and that *SAM*2 deletion can lead to an industrially relevant increase in lactic acid production.

P2.3
The HDAC inhibitor SAHA synergistically stimulates the cytotoxic effect induced by Parthenolide in MDA-MB231 cells

D. Carlisi[1], A. D'Anneo[2], M. Lauricella[1], G. Buttitta[2], S. Emanuele[1], R. Martinez[2], R. Vento[2], G. Tesoriere[2]
[1]Dept of Experimental Biomedicine and Clinical Neurosciences (BioNec), [2]Dept of Biological, Chemical and Pharmaceutical Sciences and Technologies (Stebicef), [1,2]Laboratory of Biochemistry, Polyclinic, University of Palermo,Italy

We showed that the sesquiterpene lactone Parthenolide (PN) exerts strong cytotoxic effects on triple negative breast cancer MDA-MB231 cells. Our recent results suggest that PN exerts in these cells a cytoprotective effect, which is due to the activation of mTOR pathway. To inhibit this protective response we employ the HDAC inhibitor SAHA, which is known to prevent AKT/mTOR pathway. We show that PN activates Akt, mTOR, p70S6kinase and NRF2 while SAHA abolishes these effects. Further cell pretreatment with SAHA synergistically sensitizes the cells to the cytotoxic effect of PN. Moreover SAHA alone activates the autophagic process. The addition of PN to SAHA reduces this effect and induces apoptosis. SAHA/PN combination also inhibits DNMT1 and produces hyperacetylation of histones. Epigenetic changes caused by these effects are responsible for the increased expression of oncosuppressor gene products, such as p21 and p27 and for the decrease of Bcl2 and p65 levels. In conclusion SAHA suppresses protective response exerted by PN; PN inhibits SAHA effect on autophagy and finally SAHA/PN combination induces epigenetic modifications with changes in gene expression.

P2.4
Recruitment of MyD88 in the early phases of HSV-1 infection

M. G. Ceraolo[1], G. Siracusano[1], I. Colao[1], D. Lombardo[1], A. Mastino[1], A. Esclatine[2], M.T. Sciortino[1]
[1]Dept of Biological and Environmental Sciences, University of Messina, Messina, Italy, [2]INSERM UMR756, Université Paris-Sud-11, Faculté de Pharmacie, Châtenay-Malabry, (France)

Many studies reported that HSV-1 is able to regulate autophagy through mechanisms that are not well understood. Literature data also reported that HSV-1 is recognized by TLRs and it is known that their stimulation is responsible of autophagy activation via MyD88. In this work we want to elucidate the involvement of MyD88 in the regulation of autophagy in monocytic THP-1 cells during the early phases of HSV-1 replication. To this aim, MyD88 deficient cells were also used. The data obtained showed that MyD88 protein expression was up regulated during the early times of HSV-1 infection, as well as MyD88 transcripts. The up regulation was correlated with the increase of autophagosome formation, since its forced expression increases the number of GFP-LC3 dots in infected cells. Conversely, the impairing of MyD88 in MyD88 deficient THP-1 cells prevented the occurrence of autophagy upon HSV-1 infection. These results confirm that HSV-1 mediated autophagy is triggered by signalling events initiated by the binding of the virus to cell surface receptors through the MyD88 adaptor protein.

P2.5
Expression of the animal pro-apoptotic protein Bid in Arabidopsis thaliana

M. Crimi, A. Manara
Dept. of Biotechnology, University of Verona, Italy

The major regulators of the commitment to programmed cell death in animals cells are the protein of BCL-2 family. Among these, Bid protein is crucial for both life and death of the cells. To explore whether plant programmed cell death and animal apoptosis share similar feature and control systems, we have expressed the mouse Bid protein in *A. thaliana* plants. Both the full length Bid protein and the active truncated form tBid were utilized for the transformation of *A. thaliana* and studied for their effect in plant growth and under different stress conditions to verify the interplay with PCD in plant.

P2.6
The human RNASET2 tumor antagonizing gene as a novel stress-response gene potentially involved in microenvironment-mediated tumor suppression

M. Lualdi[1], E. Pedrini[1], L. Monti[1], M. Gariboldi[1], E. Monti[1], G. Binelli[1], R. Taramelli[1], F. Acquati[1]

[1]Dept Theoretical and Applied Sciences, Insubria University, Varese, Italy

The human RNASET2 gene encodes a highly conserved extracellular RNase belonging to the Rh/T2/S family. This gene was shown to act as a non-cell autonomous tumor suppressor *in vivo* in ovarian cancer models by recruiting cells belonging to the monocyte/macrophage lineage. On these premises, and drawing from our results showing an inverse correlation between RNASET2 levels and tumor stage, we postulated a role for RNASET2 as an "alarmin"-like molecule, involved in microenvironment-mediated tumor suppression. This prompted us to address whether this protein could be responsive to microenvironmental stress challenges. In three ovarian cancer cell lines, we observed a general trend to an increase in both the intracellular (following metabolic/oxidative stress and starvation) and the secreted form (upon hypoxia) of the RNASET2 protein. Chemical hypoxia also triggered a sub-cellular re-localization of RNASET2 to Processing-bodies. Moreover, RNASET2-silenced OVCAR3 cells showed a lower sensitivity to hypoxia than control cells, displaying higher proliferation rates. Finally, we present preliminary data suggesting a role for RNASET2 in the control of the actin cytoskeleton assembly pattern.

P2.7
Apoptosis induced by HSV-1 requires BH3-only protein Puma and p73

E. Papaianni[1,2], F. Marino-Merlo[1], C. Borner[2], A. Mastino[1,3]
[1]Dept Biological and Environmental Sciences, Univ of Messina, Italy, [2]Institute of Molecular Medicine and Cell Research, Albert Ludwigs, Univ of Freiburg, Germany, [3]The Institute of Translational Pharmacology, CNR, Rome, Italy

While a number of studies identified viral and cellular components involved in prevention of the host cell premature death by Herpes simplex viruses (HSV), the exact mechanism by which HSV trigger apoptosis remains enigmatic. We, then, focused our attention on the involvement of the mitochondrial signaling pathway in the apoptotic cell death induced by HSV-1. Results clearly demonstrated the central role of the intrinsic Bax/Bak dependent mitochondrial pathway in induction of apoptosis by HSV-1 in human monocytic cells and in mouse embryo fibroblasts (MEFs). Then, we excluded that FasL, TNF, TRAIL signaling nor necroptosis could contribute to cell death caused by HSV-1. Conversely, the BH3-only protein Puma was identified as a major mediator of virus-induced, Bax/Bak-dependent apoptosis in both human monocytic cells and MEFs. Puma was transcriptionally induced after 1-6 h of virus infection and MEFs deficient for Puma were as resistant to virus-induced apoptosis as Bax/Bak doubly deficient cells (Bax/Bak DKO). In addition, ablation of p73 rendered MEFs resistant to apoptosis induced by HSV-1, thus showing the involvement of this factor in cell death triggered by HSV-1 infection

P2.8
Involvement of TOP I and PARP inhibitors in the p53/p63-dependent cell fate decision between growth arrest and apoptosis.

D. Montariello, A. Troiano, D. Di Girolamo, V. Calabrò, P. Quesada
Dept of Biology, University "Federico II", Naples, Italy

Carcinoma cells, depending on their genetic background (p53 ^{wt} versus p53 ^{null}) trigger a p53-dependent pathway to induce cell cycle arrest and apoptosis. p63 is a member of the p53 family highly expressed in carcinoma cells of epithelial origin. p63 gene encodes two major classes of proteins: those containing a transactivating (TA) domain and those lacking it (ΔN). TAp63 proteins mimic p53 function including transactivating p53 target genes and inducing apoptosis; the ΔNp63α protein has been shown to repress p53-target genes acting as an oncogene., In our study, we combined TOP I inhibitor topotecan (TPT) with PJ34 PARP inhibitor in MCF7 p53^{wt}breast carcinoma and SCC022 p53^{null} squamous carcinoma cells. We found that in MCF7 cells: i) PJ34 reverts TPT-dependent PARP 1 auto-modification and triggers caspase-dependent PARP 1 proteolysis; ii) TPT as single agent stimulates p53 expression as well as its target p21, while in combination with PJ34 increases TAp63 and BAX protein levels ; iii) TPT stimulates p53 binding at p21 promoter, while the addition of PJ34 increments BAX promoter occupancy. *Vice versa*, in SCC022 cells TPT +/- PJ34 suppress the endogenously ΔNp63α anti-apoptotic isoform, whereas p53 transfection is necessary to induce apoptosis. We suggest that PARP inhibitor(s) act in the p53/p63-mediated threshold mechanism of cell fate decision.

P2.9
Modulation of mitochondrial Ca^{+2} and Mg^{+2} dependent F_1F_O-ATPase by nitrite

S. Nesci, V. Ventrella, F. Trombetti, A. Pagliarani
Dept. Veterinary Medical Sciences, Univ. of Bologna, Ozzano Emilia, Italy

The mitochondrial F_1F_O-ATPase revealed double-faced roles: its main role of producing ATP seems to counteract the ability to cause cell death. Indeed, under conditions of Ca^{+2} accumulation, the F_1F_O-ATPase triggers the so-called Mitochondrial Permeability Transition (MPT) a lethal cellular event. Accordingly, F_1F_O-ATPase dimers would generate similar Ca^{+2}-dependent currents to MPT pore ones [1]. The F_1F_O-ATPase is more effectively inhibited by nitrite (NO_2^-) when activated by Ca^{2+}than by the natural cofactor Mg^{+2}. In the presence of Ca^{+2} or Mg^{+2}, NO_2^- uncompetitively inhibits the enzyme with respect to ATP substrate and inhibition extent depends on the cation. While NO_2^- inhibition is not prevented by ascorbate, ruling out any S-nitrosylation, it is enhanced by hydrogen peroxide (H_2O_2). The H_2O_2–driven production of nitrogen dioxide radical ($^{\cdot}NO_2$) could cause post-translational modifications of tyrosine residues in the catalytic F_1 sector. Since NO_2^-, especially provided by Mediterranean diets, acts as a vascular endocrine nitric oxide reservoir, it may protect against MPT events under ischemia/reperfusion conditions.,*1. Antoniel M. et al. 2014. Int J Mol Sci15:7513-36.*

P2.10
Deregulation of miRNAs is linked to p21 proapototic role

M.T. Piccolo[1,2], I. Favicchia[1], F. Riccardo[3], R. Calogero[3], S. Filosa[1], S. Crispi[1]
[1]Institute Biosciences and Bioresources, CNR Naples, Italy, [2]Dept Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Italy, [3]Dept Molecular Biotechnologies and Health Sciences, University of Turin, Italy

p21 is a cyclin-dependent kinase inhibitor involved in cell cycle regulation, able to mediate growth arrest in G1 and G2 phases after stress stimuli. Depending on its cellular localization, p21 has a dual role being proapoptotic in the nucleus and anti-apoptotic in the cytoplasmic compartment. Our group has evidenced a p21 proapoptotic role in mesothelioma cell lines following a piroxicam/cisplatin combined treatment. This treatment determines up-regulation of p21 mRNA and nuclear translocation of the corresponding protein. These drugs resulted ineffective in mesothelioma cell lines not expressing p21, showing a direct link between p21 and the detected apoptosis. To analyse if apoptosis occurs in p53-independent manner we are investigating an ovarian cancer cell line carrying a mutated p53. To better investigate p21 proapoptotic role, we are analysing microRNAs (miRNAs) expression profiles using next-generation sequencing. Analysis detected several upregulated miRNAs in treated cells possibly linked to this role, confirming the importance of nuclear localization for the achievement of p21 proapoptotic function.

P2.11
Characterization of recombinant human VDAC3: a VDAC forming small pores

S. Reina[1], V. Checchetto[2], A. Magrì[1], F. Guarino[1], A. Messina[1], I. Szabo[2], V. De Pinto[1]
[1]Department of Biological, Geological and Environmental Sciences, Section of Biochemistry and Molecular Biology, University of Catania, Italy, [2]Department of Biology, University of Padova, Italy

Voltage-dependent anion channels (VDACs) are pore-forming proteins of the outer mitochondrial membrane channeling metabolites up to 3000 da [1]. The electrophysiological features of VDAC1 and VDAC2 isoforms have been intensively characterized, while precise information about VDAC3 channel properties of are still missing. Several unsuccessful experiments led to the conclusion that VDAC3 is not capable of forming channels in artificial membranes [2]. In this work we demonstrate that hVDAC3, expressed in bacteria and refolded according to the procedure used for its crystallization, forms channels with a very small conductance (90 pS in 1 M KCl) with respect to hVDAC1 (3500 pS in 1 M KCl). Moreover, in contrast to VDAC1, hVDAC3 is only slightly voltage-dependent. According to the electrophysiological data, expression of hVDAC3 in a yeast strain devoid of the endogenous porin allowed only partial recovery of the growth under non-permissive conditions. Overall, the data obtained represent a preliminary but surprising biophysical characterization of VDAC3. [1] Messina A. et al. (2012) Biochim Biophys Acta 1818, 1466-1476 [2] Xu X. et al. (1999) *JMembr Biol* 170, 89-102

P2.12
F_2-Isoprostanes play a role in Bleomycine-induced lung fibrosis

B. Stringa, D. Vecchio[1,2], B. Arezzini, M. Comporti, C. Gardi
Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy, Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachiusetts, Department of Dermatology, Harvard Medical Scool, Boston, Massachusetts

F_2-Isoprostanes (Isops) are important markers of oxidative stress in different lung pathologies. We have previously demonstrated that bleomycin (BLM)-administration to rat caused the development of pulmonary fibrosis accompanied by an increased IsoPs plasma levels. The objective of our study was to investigate the role of IsoPs in the early onset of the changes that lead to pulmonary fibrosis. Rat lung fibroblasts (RLF) were treated with BLM or IsoPs and cell proliferation, collagen synthesis and α-SMA expression were evaluated. Our results demonstrated that BLM increased both cell proliferation and collagen synthesis and enhanced IsoPs production. IsoPs treatment stimulated RLF proliferation and collagen synthesis. Immunocytochemistry analyses revealed the presence of TxA_2receptor (TP) both in fibroblasts and myofibroblasts suggesting that the effect of IsoPs may be mediated by TP. IsoPs are not only markers of oxidative stress but can act also as mediator in BLM-induced pulmonary fibrosis. These effects are mediated through the binding to TP which is present both in lung fibroblasts and myofibroblasts.

P2.13
Ferritin heavy chain silencing, ROS production and protein misfolding in K562 cells

F. Zolea, F. Biamonte, M. Di Sanzo, I. Aversa, M. Panebianco, F. Trecroci, P. Candeloro, G. Cuda
Dept Experimental and Clinical Medicine, Magna Græcia Univ., Catanzaro, Italy

The redox state of the cell is involved in many patho-physiological functions and is strictly dependent on the amount of iron in its catalytically active form. Ferritin keeps the intracellular iron in a non-toxic and readily available form, therefore playing a central role in iron and redox homeostasis. The protein is composed by 24 subunits of the H- and L-type, with structural and functional differences, coded by two different genes. To study the role of the H ferritin subunit (FHC) in the control of intracellular redox state, we knocked-down its expression in K562 cells by using the shRNA silencing. Total protein extracts from control and FHC-silenced cells were analysed by Raman spectroscopy to estimate the relative amount of alpha-helix, beta-sheet and random coil. FHC silencing caused a significant increase in random-coil content, at a level comparable to that induced by H202 treatment in control cells. ROS inhibitor and iron chelators were able to revert this protein misfolding. Our data suggest that an alteration of the H/L ratio in the ferritin composition is able to induce a severe oxidative stress in K562 cells.

O2.1
3-hydroxytyrosol protects chondrocytes against microRNA-9 increased by oxidative stress

S. Cetrullo[1], S. D'Adamo[1], A. Facchini[1,2], S. Guidotti[2,3], R.M. Borzi[3], A. Facchini[3], F. Flamigni[1]
[1]Dipartimento di Scienze Biomediche e NeuroMotorie, Università di Bologna, Bologna, Italy, [2]Dipartimento di Scienze Mediche e Chirurgiche, Università di Bologna, Bologna, Italy, [3]Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Istituto Ortopedico Rizzoli, Bologna, Italy

Increasing evidence suggests that oxidative stress may be a key pathogenic factor in age-related disorders, such as osteoarthritis (OA). Currently a potential role of autophagy is considered in the regulation of the cellular response to several stress stimuli during chronic degenerative diseases. A useful alternative to drug treatment of OA may be represented by food-derived molecules. 3-hydroxytyrosol (HT) is one of the major polyphenols present in olive oil and we supposed it as a modulator of different signalling pathways. Our data showed that HT is able to reduce cell death in immortalized chondrocyte cell line C-28/I2. Moreover HT increases markers of autophagy and the total protein level of Sirtuin-1, suggesting a possible mechanism of cytoprotection. We verified that HT abolishes the increase of microRNA-9 level induced by oxidative stress. This microRNA can target Sirtuin-1 mRNA thus modulating its expression, as verified with the anti-microRNA-9 treatment. This work was supported by FIRB (Ministero dell'istruzione, dell'Università e della Ricerca, Italy) grant RBAP10KCNS and RFO (University of Bologna).

O2.2
Induction of autophagy by mutant p53-targeting molecules in cancer cells

G. Foggetti[1], L. Ottaggio[1], G. Fronza[1], M. Masini[2], P. Masiello[2], P. Menichini[1]
[1]U.O.C. Mutagenesis, IRCCS AOU San Martino-IST, 16132-Genova, Italy, [2]Dipartimento di Patologia Sperimentale, Biotecnologie Mediche, Infettivologia ed Epidemiologia, University of Pisa, Via Roma 55, Scuola Medica, 56126 Pisa, Italy

Several studies indicate that high levels of mutant p53 (mutp53) correlate with tumor aggressiveness. Thus, it is important to understand how mutp53 is stabilized in tumors and how it can be eliminated. We have demonstrated that in cancer cells PRIMA-1, known to reactivate mutp53 and to induce apoptosis, triggered mutp53 degradation via ubiquitination correlated to cytotoxicity. Mutp53 KO cells were more sensitive than the parental ones, suggesting that the removal of mutp53 eliminate a pro-survival function of mutp53 in tumors. We also found that in cell lines carrying mutant or wild type p53 PRIMA-1 induced autophagy, a possible pathway involved in the degradation of mutp53. To study the correlation between autophagy, mutp53 degradation and cell death, we are exploring the autophagic potential of Gambogic Acid (GA). GA is used in the traditional Chinese medicine and stimulates the ubiquitin/proteasome-mediated degradation of mutp53 increasing the sensitivity of cancer cells to chemotherapy. Our findings show that GA promotes mutp53 degradation correlated with the activation of autophagy. The impact of autophagy on cell death induced by this molecule will be also discussed.

O2.3
The fine tuning of autophagy and differentiation in the skeletal muscle

P. Fortini[1], L. Garribba[1], C. Ferretti[1], E. Iorio[1], M. Cagnin[2], S. Baccarini[1], M. Falchi[1], C. Isidoro[2], E. Dogliotti[1]
[1]Dept of Environment and Primary Prevention, Istituto Superiore di Sanità, Rome, Italy, [2] Department of Health Sciences, University of Piemonte Orientale, Novara, Italy

Post-mitotic cells are expected to be provided of a tight mechanism

of genome integrity control. By using an *in vitro* skeletal muscle cell differentiation system we showed that DNA repair is down-regulated in terminally differentiated muscle cells (myotubes). Despite the accumulation of damage, myotubes are extremely resistant to the cytotoxic effects of DNA damaging agents, like ionizing radiation, alkylating agents and campthotecin. Conversely, a high sensitivity to the cytotoxicity induced by doxorubicin and menadione was observed in myotubes. Preliminary data indicate that autophagy is activated during myogenesis, as testified by immunofluorescence staining and western blotting analysis of LC3, a hallmark of the autophagosome formation. In addition, myoblasts with silencing of beclin 1 show a block of the autophagic flux associated to a reduction of the fusion index, indicating a connection between autophagy and the maturation of myocytes. An attenuation of the basal autophagy was observed in p53 deficient cells in parallel to a reduced level of muscle specific proteins, supporting a crucial role of autophagy in the accomplishment of the muscle differentiation program.

O2.4
Crosstalk between autophagy and apoptosis during HSV-1 replication: possible role of Us11 viral protein

D. Lombardo, G. Siracusano, I. Colao, M.G. Ceraolo, A. Mastino, M.T. Sciortino

Dept Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres, 98166 Messina

Autophagy and apoptosis are two of main processes involved in maintenance of cellular homeostasis as well as the innate immune defense. Herpes simplex virus type 1 is known to regulate both processes. Recent studies have demonstrated that viral gene US11 is involved in control of autophagy. In order to demonstrate if there may be a crosstalk between both processes, we enrolled HSV-1 and a mutant virus R7023, with a deletion including US11 gene, to analyze autophagy and apoptosis markers. We observed that in THP-1 wt cell lines there was an activation of autophagy after infection of mutant virus R7023, but not with HSV-1. Recent studies have shown that Beclin-1, an autophagic marker, is degraded by caspase-8, which colocalizes with p62 protein on the surface of autophagosomes membrane. Activation of caspase-8 is regulated by FLIP protein. We observed that in cells infected with HSV-1, but not with mutant virus, there was a degradation of FLIP at 24, 48, 72 h.p.i. corresponding to an activation of caspase-8, that could lead to a blockage of autophagy. Based on these data we have obtained evidences about the interplays between autophagy and apoptosis pathways during viral replication.

O2.5
mRNA degradation and its effect on cellular lifespan and apoptosis in yeast

M.R. Stirpe, V. Palermo, C. Falcone, C. Mazzoni

Department of Biology and Biotechnology Charles Darwin, Pasteur Institute – Cenci Bolognetti Foundation, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

The control of mRNA degradation is an important element in regulation of gene expression. In all eukaryotes, the decay of mRNAs usually starts with the removal of the 3′ end poly(A) tail. In the principal degradation pathway in the yeast *S. cerevisiae*, deadenylation is followed by decapping and by 5′ to 3′ mRNA degradation by the exonuclease Xrn1. We previously reported that yeast mutants in genes of the mRNA decapping pathway show premature aging and undergo programmed cell death. These traits are accompanied by elevated histone mRNA levels persisting throughout the cell cycle and defects in S-phase progression. Analyzing the data concerning the negative genetic interactions of specific genes, as well those obtained with genome wide analysis, we found that gene mutants that are lethal/sick with *lsm* genes can be clustered in functional groups such as histone/chromatine modifications, protein translation, DNA replication/repair, nuclear mRNA export, mitochondrial function/biogenesis and autophagy. We are currently studying the role of these pathways in cellular aging and apoptosis related to mRNA turn over.

3 - Genomics, Proteomics and System Biology

P3.1
New insights into protamine-like component organization in *Mytilus galloprovincialis* sperm chromatin

F. Caccavale, Q.A. Vassalli, S. Avagnano, F. Pollice, G. Speranza, , D. Drongitis, G. Guerriero, L. Fucci, J. Ausió[2], M. Piscopo[1]
[1]Dept of Biology, University of Naples Federico II, Italy, [2]Dept of Biochemistry & Microbiology, University of Victoria, British Columbia, Canada

We have analyzed *Mytilus galloprovincialis'* sperm chromatin which consists of three sperm nuclear basic proteins of the PL-type: PL-II, PL-III and PL-IV, in addition to a residual amount of the four core histones. The knowledge of *Mytilus galloprovincialis'* sperm chromatin structure, the organization of the PL-type component in chromatin and their interactions with DNA is of considerable interest because these organisms are largely utilized as bioindicator of marine pollution that could affect its state. Our results obtained by micrococcal nuclease digestion in combination with salt fractionation suggest the existence of a likely unusual organization in which there would be a more accessible location of PL-II/PL-IV when compared to PL-III and core histones. Further we used electrophoretic mobility shift assay in order to define DNA binding mode of PLII and PLIII and turbidimetric assays to determine their self-association ability in the presence of sodium phosphate. On the base of our results we propose a model of *Mytilus galloprovincialis'* sperm chromatin organization that could be useful in order to develop chromatin-based genotoxicity tests in pollution biomonitoring programs.

P3.2
Juglans regia L. genotypes identification by single nucleotide polymorphisms

L.F. Ciarmiello[1,2], A. Fuggi[1], F. Iannuzzi[1], P. Carillo[1], P. Woodrow[1]
[1]Dept DiSTABiF, Second Univ. Napoli, Caserta, Italy, [2]CRA-Tree Fruit Research Unit, Caserta, Italy

English walnut (*Juglans regia* L.) is an economically important species, for both food and timber. The consumption of its kernels has been shown to have health-promoting effects. In this study mitochondrial cytochrome oxidase subunit II (COX2) and ribosomal DNA (rDNA) gene sequences from 30 English walnut genotypes were analyzed and compared. rDNA sequences revealed the presence of 402 variations, while Cox2 intron I sequences showed 769 variable positions. Based on single nucleotide polymorphism markers of rDNA and cox2 intron I sequences, an amplification refractory mutation system was used to fingerprint 18 out of 30 walnut genotypes. Therefore, cox2 intron I region, either alone or in conjunction with rDNA, could be used effectively in identifying these walnut genotypes, and the ARMS strategy may be an important tool for providing useful information on the uniqueness of accessions within germplasm collections.

P3.3
Retrotransposon copy number variation between wild and cultivated sunflowers

F. Mascagni, T. Giordani, A. Cavallini, L. Natali

Sunflower (*Helianthus annuus*) is an important crop species of the Asteraceae family. Despite its economic relevance, a detailed structural analysis of its genome is still missing. We have investigated the repetitive fraction of the sunflower genome, especially transposable elements, which constitute a major driver of genome size increasing, are able to cause lethal mutations, and may also play a role in the epigenetic setting of the genome. We first characterized the repetitive component of a sunflower homozygous experimental line, using 454 reads, then extended our studies to 7 wild accessions and 8 cultivars using Illumina reads. Using RepeatExplorer, we obtained 288 clusters representing the most representative repeat families in a random sample of sunflower reads. The annotated clusters were collected in a library, and a phylogenetic analysis of conserved regions of LTR-retroelement protein domains was performed. This library was used as reference to be mapped with reads of all the accessions. We identified 19 clusters belonging to *Gypsy* and *Copia* superfamilies showing different redundancy between cultivars and wild accessions, possibly involved in sunflower domestication.

P3.4
The inflorescence transcriptome of *Orchis italica*, a wild Mediterranean orchid species

S. De Paolo, M. Salvemini, L. Gaudio, S. Aceto
Dept Biology, Federico II Univ., Napoli, Italy

The family Orchidaceae includes more than 25,000 species known for their beauty and adaptation to different habitats. *Orchis italica* (Orchidoideae, Orchidinae), the "naked man orchid", is one of the most abundant Italian wild orchids. Recently, molecular evolutionary studies have been addressed to dissect the molecular mechanisms regulating its flower development., As the genome of *O. italica* is not available, we assembled and analyzed the floral transcriptome of *O. italica* using a *de novo* approach. Starting from more than 100 million raw reads, we obtained 132,565 assembled transcripts clustered in 86,079 unigenes. Functional annotation assigned 45.3% of unigenes to records present in the NCBI nr protein database, 37.4% to GO terms, 18.3% to KOG groups and 8.3% to KEGG pathways. We confirmed the *in silico* expression analysis by Real Time RT-PCR on 10 selected unigenes, showing significant positive correlation. In addition to the coding transcripts, we analyzed the putative long non-coding RNAs. We examined the expression pattern of selected putative long non-coding RNAs in different tissues of *O. italica*, hypothesizing their possible functional role in the floral tissues.

P3.5
Landscape genomics reveals environmental adaptation in Turkish Brachypodium distachyon natural populations

M. Dell'Acqua[1], A. Zuccolo[1], M. Tuna[2], L. Gianfranceschi[3], M.E. Pè[1]
[1]Institute of Life Sciences, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy, [2]Department of Field Crops, Namik Kemal University, 59100, Tekirdag, Turkey, [3]Department of Biosciences, Università degli Studi di Milano, via Celoria 26, 20133 Milan, Italy

We report the use of genotyping-by-sequencing on natural populations of the monocot model*Brachypodium distachyon* to identify loci linked with environmental adaptation. Individuals coming from 9 Turkish natural populations were characterized at 16,697 SNP loci and analysed with population genetics, landscape genomics and genome wide association approaches (GWAS). GIS systems were used to relate climatic features to sampling points. Sampled populations showed patterns of diversity not ascribable to their geographic distribution, and variations in sequencing depth showed consistent patterns at 8,072 genomic bins that resulted enriched in transposable elements. Through GWAS we discovered 35 environment-associated SNPs, 27 of which associated to 34 unique genes. The outlier loci approach reported 5.7% of the loci analysed being under selection. The canonical correlation analysis on regions with consistent presence and absence of reads showed for some bins a significant relation with the environmental variation. We argue that these methods may led to the exploitation of model species as natural probes to identify loci related to environmental adaptation of agronomic relevance.

P3.6
Comparative proteomic analysis of malignant pleural mesothelioma: focusing on the biphasic subtype

Y. Da Valle[1], F. Ciregia[1], L. Giusti[1], A. Bonotti[2], E. Donadio[1], R. Foddis[3], G. Giannaccini[1], G. Guglielmi[2], A. Cristaudo[2], A. Lucacchini[1]

[1]Department of Pharmacy, University of Pisa, Pisa, Italy, [2]Operative Unit of Occupational Medicine of the University Hospital of Pisa, Italy, [3]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy

Malignant pleural mesothelioma (MPM) is a rare and devastating primary tumor of the pleura linked to asbestos exposure. Recently, we performed a comparative proteome analysis between epithelioid mesothelioma (E-PM) and hyperplasia tissue biopsies. In this study we extended the comparative proteomic analysis to the biphasic mesothelioma (B-PM) characterized by a combination of elements of epitheliod and sarcomatoid subtypes. Tissue biopsies were obtained from 25 patients and classified after histological examination in B-PM, hyperplasia and E-PM. Protein patterns were analyzed and spots of interest were identified by NanoLC-ESI-MS/MS analysis and validated by western blot analysis or commercial ELISA kits. 27 proteins that showed a fold variation≥2 were identified. Likely to E-PM the different expression of prelamin, vimentin and calretinin was observed in B-PM samples with respect to hyperplasia. Moreover, other proteins resulted increased in B-PM when compared both to hyperplasia and E-PM: γ-enolase, peroxiredoxin-1, S100-A11, serum amyloid P component, and serum amyloidA1. A different expression of chloride intracellular channel protein-3 and CRYAB was also observed.

P3.7
Genetic and molecular analysis of the cross-incompatibility phenomenon in maize

H. Nugrahapraja, M. E. Pè, E. Bertolini
Institute of Life Sciences, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa (Italy)

Here we present a deep-sequencing transcriptome analysis to dissect the molecular mechanism underling pollen-pistil interaction. The role of the the Gametophyte factor 1 (Ga1) locus in maize, controlling cross-incompatibility has been addressed. Analyses were carried out in the genetic background of inbred line W22, where near-isogenic lines for three different Ga1 alleles are available., Compatible and incompatible crosses produced a series of samples for testing differential gene expression in different reproductive tissues/organs (pollen, silk, ovule). A total of 70 cDNA libraries were sequenced using the Illumina technology and produced 1.2 billion single-end reads which were processed by using the Tuxedo pipeline. Gene expression analysis revealed the modulation of many tissue specific genes and the identification of novel transcripts. In addition, the differential transcription network between pollen, silks, and ovules was dissected during the pollination process. Our large data set represents a powerful tool to shed light on the genetic mechanism controlling compatible and incompatible pollination.

P3.8
From genome to phenome and back: understanding the high metabolic versatility of *Burkholderia cepacia*complex

E. Perrin[1], M. Fondi[1], I. Maida[1], V. Orlandini[1], E. Bosi[1], A. Mengoni[1], M. Galardini[1], P. Vandamme[2], F. Decorosi[3], C. Viti[3], L. Giovannetti[3], S. Buroni[4], G. Riccardi[4], R. Alduina[5], G. Gallo[5], A.M. Puglia[5], A. Fiore[6], G. Giuliano[6], R. Fani[1]
[1]Department of Biology, University of Florence, Italy, [2]Laboratory of Microbiology, Dept. Biochemistry and Microbiology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium, [3]Department of Sciences for Agriculture and Food Production and Environment, University of Florence, Italy, [4]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy, [5]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy, [6]ENEA, Casaccia Res. Ctr., Via Anguillarese 301, 00123 Roma, Italy

*Burkholderia cepacia*complex (Bcc) species are able to colonize many different environments. This heterogeneous lifestyle and the consequent high metabolic versatility is accompanied by unusually large genomes, suggesting that particular genome structures and genetic content may support and explain in evolutionary terms such high metabolic diversity. Then, the purpose of this work was to provide a model of the relationships between genomes and phenotypic diversity in the 18 Bcc type strains, through a multi-level, systems biology approach. The genome sequences of these strains were obtained and assembled. Further analysis on the sequences obtained allowed the identification of peculiar patterns as concerning genes involved in pathogenesis, virulence, antibiotics resistance, plants growth promotion, nitrogen fixation and degradation of toxic agents. Large scale phenotypic characterization was also performed on these strains, adopting the Phenotype MicroArray technique. All those data were then used to perform an analysis of relationships between genome data and phenome results, to provide a first model of genome-metabolic description and differentiation of Bcc strains.

O3.1
Glucagon-like peptide-1 protects INS-1E against palmitate-mediated β-cell dysfunction: a proteomic study

F. Ciregia[1], Y. Da Valle[1], I. Piga[1], M. Cristofano[1], M. Bugliani[2], F. Syed[2], L. Giusti[1], P. Marchetti[2], A. Lucacchini[1]
[1]Department of Pharmacy, University of Pisa, Pisa, Italy, [2]Department of Clinical and Experimental Medicine, University of Pisa, Pisa Italy

Some evidences indicate that diabetes can result from pathogenic process at mitochondria level. In this work we decided to investigate the lipotoxic effect of palmitate on mitochondrial proteins and the protective effect of glucagone-like peptide 1 (GLP1). INS-1E beta-cells were treated for 24 h with palmitate and/or GLP1. After treatment Mitochondria were isolated from INS1E by differential centrifugation. Mitochondria isolation was verified by electron microscopy, western blot analysis and enzymatic assay and extracted mitochondrial proteins analyzed by two proteomic approaches (2DE-MS/MS spectrometry and shotgun proteomics). About 660 protein spots were obtained after 2DE separation. Proteins differentially expressed obtained from two proteomic approaches after comparison of different classes, were functionally analyzed using the Ingenuity Pathways Analysis software with the aim to determine the predominant canonical pathways and interaction network involved. The analysis revealed a specific association of the proteins altered by palmitate and/or GLP1 with the specific category of "Mitochondrial Dysfunction". Our results open a future perspective to move to the study of human biological samples.

O3.2
Modelling in the cold: genome scale metabolic reconstruction and constraints-based modelling of the Antarctic bacterium *Pseudoalteromonas haloplanktis*TAC125

M. Fondi[1,2], I. Maida[1], E. Perrin[1], A. Mellera[1,2], S. Mocali[3], E. Parrilli[4], M. L. Tutino[4], P. Liò[5], R. Fani[1,2]
[1]Laboratory of Microbial and Molecular Evolution, Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino (Firenze), [2] ComBo, Florence Computational Biology group, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino (Firenze), [3]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Agrobiologia e la Pedologia (CRA-ABP), Piazza d'Azeglio 30, 50121 Firenze, Italy, [4]Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario M. S. Angelo, Via Cintia, I-80126, Naples, Italy, [5]Computer Laboratory, Cambridge University, William Gates Building 15, JJ Thomson Avenue, Cambridge, United Kingdom

The Antarctic strain *Pseudoalteromonas haloplanktis* TAC125 is one of the model organisms of cold-adapted bacteria and is currently exploited as a new alternative expression host for numerous biotechnological applications. Here, we investigated several metabolic features of this strainthrough *in silico* modelling and functional integration of –omics data. A genome-scale metabolic model of *P. haloplanktis*TAC125 was reconstructed, encompassing information on 721 genes, 1133 metabolites and 1322 reactions. The predictive potential of this model was validated against a set of experimentally determined growth rates and a large

dataset of growth phenotypic data. Furthermore, evidence synthesis from proteomics, phenomics, physiology and metabolic modeling data revealed possible drawbacks of cold-dependent changes in gene expression on the overall metabolic network of *P. haloplanktis*TAC125. These included, for example, variations in its central metabolism, amino acids degradation and fatty acids biosynthesis. The genome scale metabolic model described here is the first one reconstructed so far for an Antarctic microbial strain. It allowed a system-level investigation of variations in cellular metabolic fluxes following a temperature downshift. It represents a valuable platform for further investigations on *P. haloplanktis* TAC125 cellular functional states and for the design of more focused strategies for its possible biotechnological exploitation.,

O3.3
Evolution of regulatory networks in *Sinorhizobium meliloti* is species- and replicon-specific

M. Galardini[1,2], G. Spini[3], M. Rossi[1], B. Roncaglia[1], A. Bani[1], G. Bacci[1], F. Pini[4], M. Brilli[5], E.G. Biondi[4], A. Mengoni[1], M. Bazzicalupo[1]

[1]Department of Biology, University of Florence, Florence, Italy, [2]EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom, [3]Department of Agricultural Biotechnology, University of Florence, Florence, Italy, [4]Interdisciplinary Research Institute USR3078, CNRS-Université Lille Nord de France, Villeneuve d'Ascq, France, [5]Edmund Mach foundation/CRI - Functional genomics - San Michele all'Adige, Trento, Italy

The information contained in a genomic sequence goes beyond the mere presence of coding sequences: the additional level of complexity added by the regulatory features allows organisms to exercise a precise control over the temporal expression of gene products and corresponding phenotypes. Recent studies have pointed out the importance of regulatory networks plasticity in bacterial adaptation and evolution but the evolution of such networks within and outside the species boundary is still obscure tough. Here we study the plasticity of the regulatory network (the pan-regulome) within and outside the *Sinorhizobium meliloti* species, looking for the presence of 46 TFs binding motifs in 51 strains and 5 additional rhizobial species.. Results showed that the evolution of the regulatory network, which incorporate a dispensable regulon for each TF, is correlated with both promoter regions and dispensable genome evolution inside the species, indicating that bacterial regulatory networks are also greatly influenced by dispensable genome dynamics.

O3.4
The interactome of human VDAC3 revealed in HeLa cells by affinity purification tag technique

A. Messina[1], A. Magrì[1], S. Reina[1], F. Guarino[1], L. Leggio[1], R.R. Ramsay[2], V. De Pinto[1]

[1]Dept. Biol., Geol. and Envir. Science, Sect. Biochemistry and Molecular Biology, Univ., Catania, Italy and National Institute of Biostructures and Biosystems, Sect. of Catania, Italy, [2]Biomedical Sciences Research Complex, Biomolecular Center, Univ. St Andrews, UK

The voltage-dependent anion channel (VDAC) is a small family of integral mitochondrial outer membrane proteins whose role is to allow the flow of hydrophilic metabolites between the mitochondrion and the cytosol. Three different VDAC isoforms have been characterized in higher eukaryotes. VDAC3 is considered the more elusive component of the family because of its relatively low abundance and because it behaves differently from the other isoforms in functional assays. To explore its functional interactions, we have established a stable cell line expressing a fluorescent and dual-tagged construct of VDAC3. In addition to live cell imaging, the proteins interacting with VDAC3 have been separated by tandem-affinity purification and 2D gel electorphoresis and identified by tandem mass spectrometry. Cytosolic proteins associated with VDAC3 include tubulins and cytoskeletal proteins, stress sensors, chaperones and and redox-mediating enzymes such as glutathione transferase. These associations are an initial indication that VDAC3 acts as a docking site for cytosolic proteins influencing mitochondrial movment and redox function [Messina A. et al., Mol. BioSystems 2014 [Epub ahead of print].

O3.5
Improvement of the nutritional potential of tomato fruits through pyramiding of favorable alleles

M. M. Rigano[1], A. Raiola[1], G. C. Tenore[2], D. M. Monti[3], R. Del Giudice[3], L. Frusciante[1], A. Barone[1]

[1]Dept. of Agricultural Sciences, Univ. of Naples Federico II, Via Università 100, 80055 Portici (Naples), Italy, [2]Dept. of Pharmacy, Univ. of Naples Federico II, Via D. Montesano 49, 80131, Naples, Italy, [3]Dept. of Chemical Sciences, Univ. of Naples Federico II, Complesso Universitario M.S.Angelo, via Cinthia 4, 80126, Naples, Italy

Solanum lycopersicum represents an important source of antioxidants and other bioactive compounds. The main aim of our study was to improve cultivated tomato varieties by increasing antioxidant level in the fruits. We previously identified two genotypes carrying loci from the wild species *Solanum pennellii* that increase fruit ascorbic acid and phenolics content. Novel tomato lines were obtained by pyramiding these selected genotypes. Pyramided lines revealed a significant increase of total phenolics, of phenolic acids, of ascorbic acid and of total antioxidant activity compared to parental lines. In addition, we analyzed the activity of tomato fruit extracts on the proliferation of normal and cancer human cells. Tomato extracts obtained from the pyramided lines had no effect on normal cells while exhibited a selective cytotoxic effect on aggressive cancer cells. Therefore, the present study demonstrates that it is possible to incorporate favorable wild alleles in the same genetic background in order to obtain genotypes with higher nutritional value.

4 - Chromosome Biology, Cell Division and Cell Cycle

P4.1
Molecular and cytogenetic characterization of cell lines mutant in the dyskerin gene

P. Leopardi, F. Marcon, E. Siniscalchi, R. Crebelli
Dept of Environment and Primary Prevention, Istituto Superiore di Sanità - Rome (Italy)

Dyskeratosis congenita is an inherited rare disease mainly associated with mutations in the Dyskerin (DKC1) gene, codifying for a nucleolar protein having different functions in telomerase stabilization, ribonucleoprotein biogenesis, rRNA processing. Recently, a new important role has been described in the modulation of spindle formation and mitotic progression. To evaluate the effects of DKC1 mutation on chromosome stability, two lymphoblastoid cell lines characterized by a point mutation (T66A) in DKC1 were selected. A 47 yrs female mutant cell line and that from her 19 yrs son were set up in parallel with two lymphoblastoid cell lines from healthy donors. Chromosome segregation was analyzed, using cytogenetic analysis of cytokinesis-blocked cells, together with molecular fluorescent techniques. FISH analysis, performed using a DNA centromeric probe to the X chromosome, highlighted significant increases in tetraploidy frequency in both mutant cell lines compared to the *wt* ones, and in aneuploidy frequency specifically in the male mutant cell line. Tetraploidization has been proposed as an intermediate step toward aneuploidy in human cancer progression. The DKC1 mutation, giving rise to an X-linked syndrome, could determine sex-mediated effects, due to the hemizygous gene condition in the male genome, where it could induce stronger molecular and cytogenetic defects, than in the female one.

P4.2
$p14^{ARF}$ reduces aneuploidy induced by CENPE post-transcriptional silencing

L. Veneziano, V. Barra, L. Lentini, A. Di Leonardo
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

The Spindle Assembly Checkpoint (SAC) is a cellular surveillance mechanism that ensures faithfully segregation of chromosomes. Weakening the SAC by reduced expression of some of its components induces chromosome instability and aneuploidy, both hallmark of cancer cells. Previously, we found that $p14^{ARF}$ could be involved in a pathway that restrict proliferation of aneuploidy cells. Centromere Protein E (CENPE) is a crucial component of the SAC and facilitates kinetochore microtubule attachment required to achieve and maintain chromosome alignment.We found that CENPE depletion by RNAi (resembling haploinsufficiency) induced widespread aneuploidy, mainly hypodiploidy, in near diploid HCT116 cells and in primary human fibroblasts (IMR90). Aneuploid cells were scored up to two weeks after CENPE depletion. In contrast, $p14^{ARF}$ ectopic expression in CENPE depleted HCT116 cells decreased the number of aneuploid cells suggesting that $p14^{ARF}$ expression could counteract aneuploidy in tumor cells likely by activating a pathway p53 controlled.

P4.3
Importin beta as a global regulator of mitosis

A. Verrico[1], E. Di Cesare[1], E. Gilistro[1], S. Moroni[1], A. Miele[1], R. De Santis[3], A. Rosa[3], V. de Turris[2], P. Lavia[1]
[1]CNR Institute of Biology, Molecular Medicine and NanoBiotechnology, Rome Italy, [2]IIT Italian Institute of Technology - Center for Life Nanoscience, Rome, Italy, [3]Department of Biology and Biotechnology, Sapienza University, Rome, Italy

Importin beta, the main vector of protein nuclear import in interphase and a major effector of the GTPase RAN, acts in multiple aspects of mitosis. Because importin beta is overexpressed in many cancer types, we have sought to gain insight into its mode of action. In addition to established roles in mitotic spindle assembly in model systems, we have identified novel importin beta-dependent processes and regulatory mechanisms. First, our recent experiments pinpoint distinct roles of importin beta in negative regulation of microtubule (MT) nucleation and MT dynamic instability in mammalian cells. Using newly generated inducible cell systems, we find that these roles are exerted via distinct interactions with downstream targets. Second, we show that Importin beta-dependent dynamic interactions with NUP358/RANBP2, a large nucleoporin with SUMO E3 ligase activity, underlie some of these processes. Finally, we suggest a new role of NUP358/RANBP2 in modulating the localization of Aurora-B during mitosis. Together these results contribute to clarify novel mechanism(s) of importin beta as a global regulator of mitosis.

O4.1
Involvement of ERK1/2 in cell cycle regulation during Herpes Simplex Virus replication

I. Colao[1], G. Siracusano, M.G. Ceraolo[1], D. Lombardo[1], A. Mastino[1], V. Hukkanen[2], M.T. Sciortino[1]
[1]Dept Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres, Messina, [2]Dept Virology, University of Turku, Kiinamyllynkatu 13, FIN-20520, Tuku Finland

Introduction and objective: In our work we have investigated the ability of HSV-1 to modulate the G1/S phase transition and the possible recruitment of ERK1/2 proteins in this regulation. Materials and Methods: we enroll flow citometry, western blot, Real-Time PCR, chemical inhibition of ERK1/2 and construction of transfectants as dominant negative for ERK1/2 (dnERK). Results: HSV-1 displayed an increase of the S-phase levels in infected HEp-2 cells, at 9 and 24 hours. These data were not consistent in HeLa cells, suggesting an early regulation of S-phase entry during viral replication in cell type dependent manner. Remarkable was the ability of HSV-1 to control the phosphorylated status of cyclin E and cdk2 proteins and the recruitment of ERK1/2 proteins in infected cells. Finally studies carried out using chemical inhibitor and HEp-dnERK demonstrated that the lacking of ERK1/2 activity affects the HSV replication and the phosphorylation status of Cyclin E/cdk2, suggesting the key role of ERK1/2 in viral replication and S-phase initiation. Conclusion: These data add a new information about the importance of cell cycle regulation in the contest of HSV-1 infection.

O4.2
Aurora-A kinase inhibition: mitotic effects and implications for anti-cancer strategies

I. A. Asteriti[1], E. Di Cesare[1], F. De Mattia[1], V. Hilsenstein[2], B. Neumann[2], E. Cundari[1], P. Lavia[1], G. Guarguaglini[1]
[1]Institute of Biology, Molecular Medicine and Nanobiotechnology-CNR, c/o Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Italy, [2]Advanced Light Microscopy Facility, EMBL, Heidelberg, Germany

The Aurora-A kinase is a major regulator of mitotic spindle assembly and function and is overexpressed in many tumor types. Inhibitors of Aurora-A activity are under evaluation in cancer therapy. We previously showed that inactivation of Aurora-A in human U2OS osteosarcoma cells yields fragmentation of spindle poles; this might originate chromosome mis-segregation and aneuploidy, thus raising concerns on the therapeutic use of Aurora-A inhibitors. To investigate the fate(s) of cells with inactive Aurora-A we have coupled high resolution and high-throughput microscopy analysis of single cells treated with the Aurora-A inhibitor MLN8237, currently under phase-I/III clinical trials. The single cell analysis in live U2OS cells enabled us to depict multiple affected mitotic processes that are differentially sensitive to the loss of Aurora-A function. Among those, we show that Aurora-A is required for the correct orientation of the mitotic spindle. We also observe that MLN8237 treatment, even in high doses, fails to induce efficient elimination of dividing cells. Instead, depending on the concentration, MLN8237 can induce mild or massive aneuploidy, hence contributing to set either a tumor-inducing or a tumor-suppressing condition, respectively. These

results pinpoint pleiotropic functions of Aurora-A and draw attention to the variability of cellular responses to its inactivation, representing a potential caveat in the use of Aurora inhibitors in anti-cancer therapy.

O4.3
SMC1B, a new mitotic-specific cohesin component, is involved in gene expression

L. Mannini[1], V. Quarantotti[1], F.Cucco[1,2], C. Amato[1], A. Frattini[3,4], A.Musio[1]

[1] Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Pisa, Italy, [2] Dipartimento di Biologia, Università di Pisa, Italy, [3] Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Milan, Italy, [4]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi dell'Insubria, Varese, Italy

Cohesin complex is composed by four evolutionary conserved subunits, SMC1A, SMC3, RAD21 and STAG. Cohesin plays a pivotal role in sister chromatid cohesion and faithful chromosome segregation in both mitosis and meiosis. SMC1β is a specific meiosis cohesin subunit in germ cells and it is essential for the assembly of chromosome axes and chromatin loop structures. To date the role of SMC1β in somatic cells has not investigated. To address this issue we assayed the expression of *Smc1β* in different mouse tissues and we found that it is expressed also in somatic cells. Thereafter, we showed that SMC1B is a member of core mitotic cohesin since it interacts with the other mitotic cohesin subunits. Finally, the inhibition of *SMC1β* by RNA interference led to gene expression dysregulation. All together these results indicate that SMC1β is novel member of a mammalian mitotic cohesin and it takes a part in the regulation of gene expression.

O4.4
The sliding behaviour of horse chromosome 11 centromere

A. Mazzagatti, FM. Piras, SG. Nergadze, E. Belloni, F. Cerutti, C. Badiale, E. Giulotto, E. Raimondi

Dept Biology and Biotechnology "L. Spallanzani", Univ. of Pavia, Pavia, Italy

The mammalian centromeres are characterised by long arrays of satellite DNA tandem repeats that hamper their molecular analysis. Equid species are exceptional in this regard; indeed, we discovered that horse chromosome 11 and a number of donkey and zebras centromeres are completely devoid of satellite DNA. This feature makes the species belonging to the genus Equus a unique biological model system to dissect centromeric function. We set up a high resolution immunoFISH technique to analyse, at the single molecule level, the interactions between centromeric single copy DNA and centromeric proteins. Here we report on single molecule analysis of the satellite-less centromeric domain of horse chromosome 11 from six unrelated horses. Previous ChIP-on-chip experiments showed that each individual exhibited a different arrangement of CENP-A binding domains. Immuno-FISH results demonstrated that each homologous chromosome 11 can carry a distinct "positional allele". We defined at least eight functional alleles spanning a 535 kb region. We conclude that the centromeric domain of horse chromosome 11 is characterized by great positional variation giving rise to "epigenetic polymorphism".

O4.5
AKTIP, an E2 variant enzyme that interacts with lamin, is required for correct telomere maintenance

R. Burla[1,2], M. Carcuro[1,2], M. La Torre[1,2], G. D. Raffa[1,2], A. Galati[1,2], D. Raimondo[3], A. Rizzo[4], L. Ciapponi[1,2], G. Cenci[1], E. Cundari[5], A. M. Biroccio[4], S. Cacchione[1,2], M. Gatti[1,2,5], I. Saggio[1,2,5]

[1]Dipartimento di Biologia e Biotecnologie, Sapienza, Università di Roma, 00185, Italy, [2] Istituto Pasteur Fondazione Cenci Bolognetti, Sapienza, Università di Roma, 00185, Italy, [3]Dipartimento di Fisica, Sapienza, Università di Roma, 00185, Italy, [4]Istituto Nazionale Tumori Regina Elena, Rome 00144, Italy, [5] Istituto di Biologia e Patologia Molecolari del CNR, Sapienza, Università di Roma, 00185, Italy

Telomeric DNA challenges cell metabolism requiring the shelterin complex and the telomere accessory factors for chromosome protection and correct replication. We report on AKTIP, an ubiquitin E2 variant enzyme that we have identified as required for mammalian telomere metabolism. AKTIP was investigated on the basis of its homology with the *Drosophila* telomere protein Peo. AKTIP interacts with TRF1 and TRF2 and with telomeric DNA. Loss of AKTIP results in fragile telomeres, which have been associated with telomere replication problems. In doubly depleted TRF1/AKTIP cells, the TRF1-induced fragile telomere phenotype was epistatic to that of AKTIP, suggesting that they are involved in a common pathway. In AKTIP depleted cells, telomeric replication is impaired and AKTIP interacts with the replication factors PCNA and RPA70. AKTIP locates at the nuclear periphery, the site of late replicating heterochromatic DNA, and interacts with lamins. In vivo, the depletion of the mouse homologue of AKTIP causes developmental abnormalities and premature death. Our results suggest that AKTIP plays a crucial role in telomere maintenance presumably acting in lamin-associated replication factories.

5 - DNA replication, Repair and Recombination

P5.1
Fine regulation of DNA damage apoptosis in *C. elegans* meiosis

A. Adamo, A. La Volpe
Institute of Biosciences and Bioresources,CNR, Napoli, Italy

In the *C. elegans* germline, by the end of pachytene, about half of the oocytes nuclei undergo physiological cell death. In addition, apoptosis is stimulated by DNA damage that can activate a checkpoint. Among the fundamental genes involved in the DNA damage checkpoint activation are *hus-1*, the p53 orthologue *cep-1*, and *egl-1*. Recently, we have shown that, the MSH-4, MSH-5 and ZHP-3 procrossover factors, that are necessary for crossover formation, are also required for DNA damaged induced apoptosis. In fact, in the absence of these proteins, both DNA damage apoptosis induced by IR, or by failure in DNA repair due to mutations in genes such as, *brc-1* or *fcd-2*, is abrogate (Adamo et al., 2008; Adamo et al., 2010; Silva et al., 2013). However apoptosis enhancement in some other DNA repair mutants, such as *rad-51* and *him-6*, is not suppressed by mutations in above mentioned procrossover factors. We are investigating whether other key factors of meiosis may be necessary for damage dependent apoptosis in *C. elegans*. We already have evidences that COM-1 (homolog of CtIP/Sae2/Ctp1), a crucial regulator of meiotic DSB repair pathway choise, is necessary for damage dependent apoptosis.

P5.2
Myc suppression of DNA double-strand break repair

S. Ambrosio[1], S. Amente[2], G. Di Palo[2,3], G. Napolitano[1], M. Faretta[3], I. Dellino[3], L. Lania[2], P.G. Pelicci[3],B. Majello[1]
[1]Dept. of Biology, Univ. Federico II, Naples, Italy, [2]Dept. of Molecular Medicine and Medical Biotechnologies, Univ. Federico II, Naples, Italy., [3]Dept. of Experimental Oncology, European Institute of Oncology, Milan, Italy

The Myc family members are regulators of cell proliferation and their expression is associated with tumors. Overexpression of Myc enhances chromosomal aberration and genetic instability. However, the mechanism(s) involved remains elusive. It is possible that Myc-induced genetic instability may result from increased DNA damage and/or reduced DNA repair. To determine how overexpression of Myc proteins (c-Myc and N-Myc) influences DNA repair, we employed the inducible AsiSI-ER cellular system to generate, by 4-OHT administration, DSBs at defined chromosomal loci in three human cell lines, U2OS, MCF10A and TET21N, in the presence or absence of overexpressed Myc proteins. After DSB induction and removal of 4-OHT, both c-Myc and N-myc overexpression reduced cellular recovery as monitored by cell viability and expression of P-p53. Appearance of DSB markers (γH2AX and NBS1) was monitored by ChIP and ChIP-seq approaches. We found that both c-Myc and N-Myc do not increase accumulation of γH2AX and NBS1 at DSB sites, rather, the disappearance of γH2AX and NBS1 was slower in Myc-overexpressed cells. These findings suggest that enhanced expression of Myc suppress efficiency DNA repair.

P5.3
Interplay between Werner syndrome protein (WRN) and the ATR-checkpoint in preserving genome integrity at common fragile sites

G. Basile, G. Leuzzi, P. Pichierri , A. Franchitto
Dept. of Environment and Primary Prevention, Istituto Superiore di Sanità, Roma, Italy

Werner syndrome is a cancer-prone disease caused by a mutation in the *WRN* gene. Common fragile sites (CFS) are genomic loci especially prone to breakage upon mild replication stress. We previously implicated WRN in preventing CFS expression acting with the ATR-pathway, but with unknown mechanisms. Here, we establish a novel function of WRN in mediating CHK1 activation under moderate replication stress, like that inducing CFS. We provide evidence that CHK1 phosphorylation relies on the ATR-mediated phosphorylation of WRN but not its helicase activity. By using analysis of replication fork dynamics, we show that loss of WRN checkpoint mediator function, as well as of WRN helicase activity, hampers replication fork progression, and lead to new origin activation to recover from replication slowing upon replication stress. Furthermore, we demonstrate that bypass of the WRN checkpoint mediator function through the overexpression of a phospho-mimic CHK1 form is sufficient restores the wild-type fork progression and CFS stability. Therefore, our findings suggest a novel role for WRN in contributing to checkpoint activation upon mild replication stress that regulates CFS stability.

P5.4
Effect of the telomeric G-quadruplex ligand RHPS4 in NHEJ-proficient (M059K) and -deficient (M059J) glioblastoma cell lines

F. Berardinelli, M. De Piano, C. Tanzarella, A. Sgura, A. Antoccia
Dept of Sciences, University of Rome "Roma Tre"

G-quadruplex (G4) interacting agents are a class of ligands able to bind to and stabilize secondary structures located in genomic G-rich regions such as telomeres. Stabilization of G4 leads to telomere architecture disruption and consequent detrimental effects on genome stability and cell proliferation, since dysfunctional telomeres are recognized as DSBs. To analyze the relationship between dysfunctional telomeres and the function of non-homologous end-joining pathway (NHEJ), M059K and M059J human glioblastoma cells (proficient and deficient in DNA-PKcs activity, respectively) were exposed to RHPS4, one of the most effective and studied G4 ligands with a very high specificity for telomeric G4., Very preliminary data will be presented regarding the impact of micromolar concentrations of RHPS4 on cell growth reduction, cell cycle, phosphorylation of H2AX histone, induction of telomere-induced dysfunctional foci and cytogenetic effects.

P5.5
A cell culture comparative biology approach to study mechanisms of genome stability and their relevance for species longevity: a newer interpretation of DNA damage induced nuclear foci.

E. Croco[1,3], S. Marchionni[1], M. Bocchini[1],T. Stamato[4], M. Malaguti[3], S. Hrelia[3], C. Sell[2], A. Lorenzini[1]
[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, [2]Drexel University, Philadelphia, PA, U.S.A., [3]Department for Life Quality Studies, University of Bologna, [4]Lankenau Institute for medical Research, Wynnewood, PA, U.S.A.

The DNA-damage response (DDR) initiates cellular processes that can lead to DNA repair, senescence or apoptosis. Differences in DDR efficiency may contribute to species-specific differences in lifespan and partially explain the exceptional longevity of some species. To better understand the differences in genomic stability between species, in fibroblast cultures from several mammals, we have examined the appearance of micronuclei and the recruitment of 53BP1 in nuclear structures termed foci, after genotoxic insults represented by Etoposide and Neocarcinostatin. Quantification of 53BP1 foci formation together with micronuclei appearance up to three days after damage showed that cells from long-lived species appear to be better equipped in controlling progression into the cell cycle. This capacity may be the consequence of a better ability to detect DNA damage. We propose a newer interpretation of nuclear foci. They do not simply represent the presence of DNA damage but rather the cell awareness of it. We suggest that a key element for species longevity is the capacity to detect damage and to take the necessary time to make an accurate choice between repair, senescence or apoptosis.

P5.6
A rapid and cost-effective assay to measure functional activity of Base Excision Repair enzymes in cell extracts

C. Cerasaro, M. Bignami, F. Mazzei
Cancerogenesi sperimentale e computazionale, Dipartimento Ambiente e connessa prevenzione primaria, Istituto Superiore di Sanità, Roma, Italy

The genomic integrity is essential for cell survival, but spontaneous modifications and highly active radicals continuously threaten it. Cells have developed a number of mechanisms to counteract these insults and to repair damage to DNA. Defects of DNA repair capacity has been found associated to several pathologies and to aging, while overexpression of some DNA repair genes has been found related to drug- and radio-resistance effects. In both cases monitoring of DNA repair capacity is a useful tool and several *in vitro* and *in vivo* assays have been proposed to measure it. Here we described an easy-to-use and low-cost method where cell-free extracts are incubated with a molecular beacon DNA substrate, containing a single lesion. The method is based on the use of a streptavidin platform to immobilize the biotinylated DNA, of on-site reactions in multiwell plates, and then on fluorescence data collection from cleaved beacon. Fully removal of all unbound components from the plates is obtained. The method, which has been set up for monitoring OGG1 and APendonuclease1 activity, has been proven to be highly sensitive and reproducible and is suitable for high throughput screening assays.

P5.7
Oncogenic disruption of promyelocytic leukemia nuclear bodies results in defective DNA double strand breaks sensing and repair

D. Cilli[1], I. Pallavicini[2], A. Antoccia[1], F. Berardinelli[1], P. Ascenzi[1], S. Minucci[2], C. Nervi[3], A. di Masi[1]
[1]Dept of Sciences, University of Roma Tre, Rome, Italy, [2]Dept of Experimental Oncology, IEO, European Institute of Oncology, Milan, Italy, [3]Dept of Medico-Surgical Sciences and Biotechnologies, University of Rome "La Sapienza", Latina, Italy

Promyelocytic leukemia nuclear bodies (PML-NBs) are implicated also in the DNA damage response (DDR). Indeed, many proteins involved in the DNA double strand break (DSB) damage response localize to PML-NBs. The PML-RARα fusion protein, generated by the t(15;17) chromosomal translocation occurring in >95% of acute promyelocytic leukemia (APL) patients, interferes with PML-NBs assembly. The aim of the present work is to shed light on the role of PML-NBs in the DSB sensing and repair. We found that PML-RARα expression causes a delayed repair of X-ray-induced DSBs, and affects the proper activation of the ATM kinase and related substrates (H2AX, NBN, CHK2). Remarkably, Sp100, one of the constitutive component of PML-NBs, seems to play a role in the PML-NB-dependent DDR, since it interacts with NBN and localizes on the DSB sites. Cycle cycle, apoptosis and chromosomal damage have been analysed to further characterize the role of PML-RARα in the genomic stability. Results obtained in APL cell lines were confirmed in primary cells from patients and in an APL mouse model, thus supporting that PML-RARα expression is per se responsible for the defective DSB sensing and repair.

P5.8
An oxidized dNTPs pool and aberrant repair synthesis contribute to GAC/CTG repeat instability

P. Cilli[1], I. Ventura[1], A. Minoprio[1], E. Meccia[1], A. Martire[2], P. Popoli[2], M. Bignami[1], F. Mazzei[1]
[1]Department of Environment and Primary Prevention, [2] Department of Drug Safety and Evaluation, Istituto Superiore di Sanità, Roma, Italy

Expansion of trinucleotide CAG/CTG repeats (TNRs) is the cause of several neurodegenerative diseases including Huntington's disease (HD). It has been proposed that, in the presence of oxidative stress, base excision repair (BER) inefficiency and/or disequilibria among BER enzymes might favour TNR expansion. We confirmed a major role of Polymerase β (POL β) and FEN1 in affecting TNR stability and identified Endonuclease III and an oxidized dNTPs pool as novel players in this process. During repair synthesis POL β can incorporate 8-oxodGMP leading to the formation of 8-oxoG:C and 8-oxoG:A mismatches in these sequences. These are substrates for the OGG1 and MUTYH DNA glycosylases that would create incisions on opposite DNA strands from where elongation of the two strands might occur. In a mouse model for HD, the R6/2 animals, we observed an unbalance in POL β and FEN1 expression in brain areas affected by the disease (striatum and motor cortex). OGG1 and MUTYH proteins were equally present in these areas. I*n vitro* and *in vivo* data support a model where an oxidized dNTPs pool together with aberrant repair synthesis contribute to TNR expansion in non-replicating cells.

P5.9
Mycobacterium tuberculosis:investigation of the adaptive response

P. di Pasquale, F. De Maria, A. Duilio
Dept Chemical Sciences, University of Naples Federico II, Naples, Italy

Nowadays the increase of human migration and the development of extensively drug-resistant strains led to a new diffusion of the tuberculosis, one of the most ancient infection in the world [1]. Thereby, proteins involved in DNA repair and protection represent excellent therapeutic targets because of the fundamental role they play in bacterial survival and due to the absence of homologues in humans [2]. The work was principally addressed to obtain a comprehensive description of an adaptive response component to methylation stress. *In silico* investigations revealed the presence of FadE8, a putative DNA protection protein homologous to *E.coli* AidB, a DNA protective protein [3]. The corresponding gene was expressed in *E.coli*. The recombinant FadE8 protein was structurally and functionally characterized. A Multiple Reaction Monitoring (MRM) tandem MS procedure was used to qualitative and quantitative analyze DNA modifications. Through this approach, *E.coli* and MTB systems were eventually compared and the functional roles of homologue proteins involved were highlighted. [1] Napier RJ, 2012, Future microbiology [2] Dos Vultos T, 2009, FEMS microbiology reviews [3] Rippa V, 2011, DNA repair

P5.10
p21 regulates the affinity of PARP-1 for damaged DNA

I. Dutto[1], M.V. Sukhanova[2], M. Tillhon[1], O. Cazzalini[3], L.A. Stivala[3], A.I. Scovassi[1], O. Lavrik[2], E. Prosperi[1]
[1]Inst. of Molecular Genetics of CNR, Pavia, Italy; [2]Inst of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia; [3]Dept of Molecular Medicine, University of Pavia; Pavia, Italy

The poly(ADP-ribose) polymerase 1 (PARP-1) is able to recognize damaged DNA and to recruit DNA repair, mainly base excision repair (BER), factors, to the lesion site. We have previously shown that p21 interacts with the automodification/DNA-binding domain of PARP-1 and that PAR (poly(ADP-ribose)) synthesis by PARP-1 was higher in human p21-/-parental cells damage. To investigate the role of p21 as regulator of PARP-1 activity, we have analyzed its impact on PARP-1 association to BER enzymes, such as DNA pol β, and DNA repair intermediates, with photoaffinity labeling technique by using recombinant purified proteins or nuclear cell extracts. The results indicate that p21 stimulates PARP-1 binding to DNA damage sites and that the interaction between PARP-1 and p21 occurs mainly in the presence of automodified PARP-1, although observed also when PARP-1 activity is inhibited by Olaparib. These results suggest that p21 modulates PARP-1 affinity to the DNA damage site thereby promoting productive interaction of PARP-1 with BER factors, in order to allow efficient DNA repair.

P5.11
A novel role for the WRN exonuclease activity: processing and protection of reversed forks from MRE11-mediated degradation

C. Iannascoli, A. Minoprio, F. Mazzei, A. Franchitto, P. Pichierri
Dept of Primary Prevention and Environment, Istituto Superiore di Sanità, Rome, Italy

The WRN protein is a RECQ helicase acting on perturbed forks by promoting their repair or recovery. One of the reactions occurring at perturbed replication forks is replication fork reversal (RFR). Since WRN exonuclease and helicase enzymatic activities cooperate in fork processing *in vitro*, we wanted to investigate the role they play in RFR *in vivo*. Using nanomolar CPT doses that induce RFR but not DSBs, we found that expression of the exonuclease-dead WRN protein induced defective CHK1 activation, reduced fork speed and enhanced ssDNA formation accompanied by an earlier accumulation of MRE11 foci respect to wild-type cells. These observations are further corroborated by *in vitro* experiments, showing degradation of a model reversed fork when incubated with nuclear extracts from cells expressing exonuclease-dead WRN. Furthermore, we show that aberrant or defective processing of regressed forks led to enhanced chromosomal instability. Altogether these results, suggest that WRN-exonuclease is required, alone or in collaboration with other exonucleases, for proper processing of reversed forks, counteracting their degradation and ensuring a proper CHK1 activation and genome integrity.

P5.12
A novel role for the Werner helicase-interacting Protein 1 in protecting genome integrity during DNA replication

G. Leuzzi, P. Pichierri, A. Franchitto
Dept. of Environment and Primary Prevention, Istituto Superiore di Sanità, Roma, Italy

Werner helicase-interacting protein 1 (WRNIP1) has been originally identified as the main interactor of the Werner protein, mutated in the Werner syndrome. WRNIP1 has been found to reside in DNA replication factories and it has been proposed to be a sensor of DNA damage in yeast. However, the function of human WRNIP1 still remains elusive. Here, we report that loss of WRNIP1 determines a reduced ability to elongate ongoing forks under unperturbed replication and instability at common fragile sites (CFS), which are genomic regions prone to breakage after mild replication stress induced by low aphidicolin doses. We also found that WRNIP1, upon mild replication stress, limits the accumulation of DNA gaps counteracting MRE11-dependent degradation and RAD51 recruitment. Interestingly, loss of WRNIP1 seems to hamper replication forks elongation after treatment with low dose of aphidicolin, resulting in firing of more replication origins. Previous data have shown that Werner helicase is a key regulator of CFS stability, our results suggest that a WRNIP1/WRN might be crucial during normal S-phase progression to avoid aberrant DNA replication, thereby protecting against DNA breakage at CFS.

P5.13
MUS81 and GEN1 nucleases are involved in DSBs formation after CHK1 inhibition

E. Malacaria, A. Franchitto, P. Pichierri
Department of Primary Prevention and Environment – Istituto Superiore di Sanità – Roma (Italy)

Under replication stress conditions, as caused by defective CHK1 activity, DSBs are the consequence of MUS81 endonucleolytic cleavage of replication/recombination intermediates arising at perturbed forks to restart stalled replication forks by promoting HR through a RAD52-mediated pathway. During recombination, MUS81-EME1 cooperates with two other nucleases, hSLX4 and GEN1 that process a variety of DNA junction substrates. We analysed the genetic relationship between MUS81-EME1 and hSLX4 or GEN1 in cells with inactive CHK1, thus during replication under pathological conditions. We show that when CHK1 activity is inhibited, MUS81-SLX4 genetic association was maintained but GEN1 is necessary for cell vitality when they are absent. Our data suggest that, under pathological conditions, hSLX4 must be removed to allow GEN1-dependent formation of DSBs during replication or late-G2 phase. Thus, our results demonstrate that MUS81 and GEN1 define two independent mechanisms to cleave intermediates formed at stressed replication forks in cells with impaired CHK1 function.

P5.14
Activity of AID driven by cell cycle specific promoters

G. Mattiuz, C. Sala, G. Saraconi, S.G. Conticello

Activation Induced Deaminase (AID) triggers all antigen-dependent antibody diversification processes by induction of DNA damage at the immunoglobulin locus (Ig) that result in different outcomes depending on the specific pathway of DNA repair that is elicited. These mutational patterns are identifiable also in non-Ig genes in mature B cell tumors. Understanding the weight of the different pathways involved could help clarifying how non-Ig genes are targeted by AID. Our working hypothesis is that AID-dependent damage at different phases of the cell cycle might trigger different outcomes and be possibly indicative of its activity on non-Ig genes. We thus placed AID under the control of promoters active ad different phases of the cell cycle, and we transduced them in chicken lymphoma DT40 cells, a cell line often used to study AID-induced hypermutation. We have then assessed their ability to trigger mutations on the Ig locus. To this aim we have developed a deep-sequencing approach that allows us to analyse the mutational status of the variable region of the Ig with a greater sensibility than previous approaches. We will thus present the effects of these constructs on the efficiency of somatic hypermutation.

P5.15
Somatic mutations patterns in colorectal cancer patients affected by MUTYH-associated polyposis

E. Meccia[5], A. Viel[1], E. Giacomini[1], E. Urso[2], M. Agostini[2], M. Genuardi[3], M.G. Diodoro[4], L. Sanchez-Mete[4], V. Stigliano[4], F. Mazzei[5], F. Grasso[5], E. Policicchio[5], A. Bruselles[5], M. Tartaglia[5], M. Bignami[5]
[1]Centro di Riferimento Oncologico, Aviano, Italy, [2]University of Padova, Italy, [3]University of Florence, Italy, [4]Regina Elena National Cancer Institute, Rome, Italy, [5]Istituto Superiore di Sanità, Rome, Italy

Germ-line mutations in the *MUTYH* DNA glycosylase associate with colorectal adenomatous polyposis (MUTYH-associated polyposis, MAP). MAP is an autosomal recessive syndrome characterized by a mutator phenotype and accumulation of somatic GC->TA transversions, most likely due to unrepaired 8oxoG/A mismatches. We aim to investigate if colorectal cancer in MAP patients progresses through MAP specific pathways and somatic mutation patterns compared to sporadic CRC. As limited information is available on the spectrum of somatic mutations occurring in CRC associated with MUTYH inactivation, we tried to address this issue through a *whole exome sequencing* approach based on a NimbleGen 64M Capture kit and subsequent sequencing using Illumina's HiSeq technology. Whole exome sequences from CRC samples of seven MAP patients were performed and compared to healthy tissues from the same patient. MAP patients were either homozygous for p.G396D (FAP 173) or p.Y179C (FAP 450) mutations or compound heterozygotes (p.Y179C/p.R235H, p.Y179C/p.G396D, p.Y179C/c.933+3A>C). This approach will allow identifying functionally relevant somatic mutations in CRC specifically associated with MUTYH inactivation.

P5.16
MUS81 phosphorylation by CHK2 is required for its efficient activation during S- and G2/M phases of cell cycle

I. Murfuni[1], G.M. Pugliese[1], S. Rinalducci[2], L. Zolla[2], A. Franchitto[1], P. Pichierri[1]
[1]Department of Primary Prevention and Environment, Istituto Superiore

di Sanità, [2]Department of Ecology and Biology – University of Tuscia – Viterbo (Italy)

MUS81 is a structure-specific endonuclease that processes DNA structures at stalled or collapsed replication forks. We recently showed that MUS81 is responsible for cleaving of RAD52-dependent structures formed at stalled forks in the absence of CHK1. Unscheduled action of MUS81 should be tightly controlled but very little is known about its regulation in human cells. Here we show that human MUS81 is phosphorylated by CK2, and by the S-phase checkpoint kinases CHK2 and CHK1. Analysis by MS/MS identified two residues, T86 and S87, which lie within the CHK2 and CK2 consensus sequences. Functional analysis on cells expressing unphosphorylable (T86A, S87A) or phosphomimetic mutants (T86D, S87D) of MUS81 revealed that phosphorylation at T86 and S87 is required for MUS81-dependent resolution of replication and recombination intermediates. Interestingly, further studies demonstrated that T86 phosphorylation could require priming modification of S87. Our results suggest a complex regulation of MUS81 function in response to replication perturbation.

P5.17

Functional analysis of the putative FHA-binding site of MUS81 during replication

A. Palma, A. Franchitto, P. Pichierri

Department of Primary Prevention and Environment – Istituto Superiore di Sanità – Roma (Italy)

The MUS81 endonuclease is involved in producing Double Strand Breaks (DSBs) at replication forks in response to replication stress induced by loss of fork-stabilizing factors or oncogene expression. In yeast, MUS81 is negatively regulated by Cds1 via its ForkHead Associated (FHA) domain, but little is known of MUS81 regulation in human cells. Interestingly, upon replication stress, CHK2 -the structural homologue of Cds1- positively regulates the biological activity of MUS81, suggesting a more complex scenario in human cells. We demonstrated that the CHK2-FHA domain is able to bind MUS81 in a pull-down assay, and that the canonical FHA binding site of MUS81 contains the phosphorylated threonine residue T159. We showed that T159A mutation increases the induction of MUS81-dependent DSBs, especially under normal replication, suggesting for a functional role of the FHA-dependent CHK2-MUS81 interaction and a dual role of CHK2 during replication stress. Further experiments are ongoing to characterize the kinase-dependent dynamics and protein-protein interaction landscape of MUS81 by Proximity Ligation Assay, while MS/MS analyses will identify novel FHA interactions of MUS81.

P5.18

Isolation of mutants suppressing fcd-2 phenotypes in Caenorhabditis elegans

P. Santonicola[2,1], A. Adamo[1], M. Germoglio[2,1], A. La Volpe[1]

[1]CNR Institute of Biosciences and Bioresources, Napoli, Italy, [2]Seconda Università degli Studi di Napoli, Caserta, Italy

Fanconi anemia (FA) is a cancer predisposition syndrome with cells sensitivity to interstrand crosslinking (ICL) agents such as cisplatin (CDDP). Genes in the FA pathway are evolutionary conserved, allowing dissection and mechanistic studies in the model system *C. elegans*. The FA pathway is involved in the choice between the high fidelity repair pathway, Homologous Repair (HR), and the error prone Non-Homologous End Joining (NHEJ). In *C. elegans* FCD-2 suppresses the use of NHEJ pathway and prevents DNA repair defects in mutant worms. Our goal is to identify genes that, when mutated, bypass the checkpoints and determine ICL tolerance/resistance in a *fcd-2* mutated background. To dissect the genetic pathways we have identified some mutants, named *clt*for Cross-linking tolerance. We are characterizing these mutants for other important phenotypes such as developmental defects, apoptosis levels, RAD-51 levels. We are transferring the mutations to a different genetic background in order to proceed in the identification of the specific loci by genetic map or genome re-sequencing.

O5.1

Colorectal carcinogenesis and the inflammatory response: a role for the MUTYH DNA glycosylase

F. Grasso[1], S. Di Meo[2], G. De Luca[3], L. Pasquini[3], M. Biffoni[3], E. Di Carlo[2], M. Bignami[1]

[1]Department of Environment and Primary Prevention, Istituto Superiore di Sanità, Rome, Italy, [2]Gabriele D'Annunzio University of Chieti, Chieti, Italy, [3]Department of Molecular Medicine and Oncology, Istituto Superiore di Sanità, Rome, Italy

8-Oxo-7,8-dihydroguanine (8-oxodG) is an oxidized base involved in mutagenesis and carcinogenesis. During replication this oxidized purine directs incorporation of C or A with almost equal efficiency, leading to GCàTA transversions. The MUTYH DNA glycosylase counteracts this mutagenic effect by removing A opposite 8-oxodG and mutations in this gene cause in humans the autosomically recessive MUTYH-associated polyposis (MAP). Since previous evidence indicated that MUTYH might influence tumorigenesis in an inflammatory background, we used *Mutyh*$^{-/-}$mice for studying the tumour susceptibility associated with an established inflammation-related model of colorectal carcinogenesis: initiation/promotion by azoxymethane and dextran sodium sulfate. Indeed the accumulation of 8-oxodG in the gastro-intestinal tract of *Mutyh*$^{-/-}$in comparison to wild-type mice was accompanied by an increased number of adenomas induced by the combined treatment. A characterization of the inflammatory response of KO mice is ongoing. The results support the pathogenic role of MUTYH inactivation in colorectal carcinogenesis and identify novel molecular features for a better understanding of MUTYH pathogenesis.

O5.2

A cell culture comparative biology approach to study mechanisms of genome stability and their relevance for species longevity: a newer interpretation of DNA damage induced nuclear foci

E. Croco[1,3], S. Marchionni[1], M. Bocchini[1], T. Stamato[4], M. Malaguti[3], S. Hrelia[3], C. Sell[2], A. Lorenzini[1]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, [2]Drexel University, Philadelphia, PA, U.S.A., [3]Department for Life Quality Studies, University of Bologna, [4]Lankenau Institute for medical Research, Wynnewood, PA, U.S.A.

The DNA-damage response (DDR) initiates cellular processes that can lead to DNA repair, senescence or apoptosis. Differences in DDR efficiency may contribute to species-specific differences in lifespan and partially explain the exceptional longevity of some species. To better understand the differences in genomic stability between species, in fibroblast cultures from several mammals, we have examined the appearance of micronuclei and the recruitment of 53BP1 in nuclear structures termed foci, after genotoxic insults represented by Etoposide and Neocarcinostatin. Quantification of 53BP1 foci formation together with micronuclei appearance up to three days after damage showed that cells from long-lived species appear to be better equipped in controlling progression into the cell cycle. This capacity may be the consequence of a better ability to detect DNA damage. We propose a newer interpretation of nuclear foci. They do not simply represent the presence of DNA damage but rather the cell awareness of it. We suggest that a key element for species longevity is the capacity to detect damage and to take the necessary time to make an accurate choice between repair, senescence or apoptosis.

O5.3

WRN phosphorylation by CDK modulates pathway choice to repair DNA double strand breaks after end-resection

V. Palermo[1], S. Rinalducci[2], M. Sanchez[3], L. Zolla[2], A. Franchitto[1], P. Pichierri[1]

[1]Department of Primary Prevention and Environment – Istituto Superiore di Sanità – Roma (Italy), [2]Department of Ecology and Biology – University of Tuscia – Viterbo (Italy), [3]Department of

Oncology and Molecular Medicine - Istituto Superiore di Sanità – Roma (Italy)

Homologous Recombination (HR) is the major pathway activated to repair DNA double strand breaks (DSBs). HR initiates with processing of the DNA ends by nucleases and helicases to form 3'-ssDNA overhang tails for subsequent repair. The end-resection process is regulated by CDK-dependent phosphorylation and involves, in yeast, the RecQ helicase Sgs1. Here, we asked whether one of the human RecQ helicases, WRN, could have a similar role., We provide evidence that WRN is phosphorylated by CDKs in vivo and in vitro and identified Serine 1133 as the major CDK phosphorylation site after DSBs. To investigate the functional role of this phosphorylation, we generated phosphomutants of WRN and evaluated end-resection upon induction of DSBs by CPT treatment. We show that S1133 phosphorylation is required for proper end-resection, CHK1 activation and recombination by single-strand annealing, while constitutively phosphorylated WRN deregulates RAD51-dependent HR. Collectively, our data suggest that WRN phosphorylation by CDK is strictly modulated to allow correct pathway choice at DBSs and activate the DNA Damage Checkpoint.

O5.4
Altered replication timing of the frataxin gene in the presence of the GAA/TTC repeat expansion

M. Stevanoni, E. Palumbo, A. Russo
Department of Biology, University of Padova, Padova, Italy

The GAA/TTC repeat expansion in intron 1 of frataxin gene is the recessive mutation responsible for Friedreich's ataxia (FRDA). We investigated whether the expanded repeat may affect the replication profile of the locus, a mid-late replicating domain. Cell lines from FRDA patients and healthy relatives were FACS sorted in 4 early-to-late S-phase fractions (S1-S4). An altered replication timing for the mutated allele was revealed by interphase FISH (replicated alleles appear as paired spots). Interestingly, in the S2 fraction of patient cells the proportion of non-replicated alleles was twice than in cells from healthy relatives ($P<0.001$). Instead, in S3 cells the replication delay appears to be bypassed. We used molecular combing to address the hypothesis that additional replication origins and/or acceleration of fork speed are involved in the recovery from a replication fork arrest at the expanded repeat. In the unsorted cell population no differences were found with respect to estimates from the normal allele and other loci (Palumbo et al 2013 Exp. Cell Res 319:3081-93). To complete the analysis, we are now characterising the replication forks firing during the mid-late S phase.

O5.5
CBP/p300-mediated acetylation of PCNA is required for its chromatin removal and degradation in nucleotide excision repair

M. Tillhon[1], O. Cazzalini[2], S. Sommatis[2], I. Dutto[1], A. Bachi[3], A. Rapp[4], T. Nardo[1], A.I. Scovassi[1], D. Necchi[5], M.C. Cardoso[4], L.A. Stivala[2], E. Prosperi[1]
[1]Institute of Molecular Genetics, National Research Council (CNR), Pavia, 27100, Italy., [2]Department of Molecular Medicine, University of Pavia, Pavia, 27100, Italy., [3]IFOM-FIRC Institute of Molecular Oncology, Milan, 20100, Italy., [4]Technische Universität Darmstadt, Darmstadt, 64287, Germany., [5]Department of Drug Sciences, University of Pavia, Pavia, Italy.

PCNA protein, a molecular platform recruiting and coordinating the activity of several DNA transaction factors, appears to be regulated by different post-translational modifications, among which acetylation. In this study, we have identified CBP, as another acetyltransferase (p300 homolog) interacting with PCNA, and found that PCNA is acetylated after UV-induced DNA damage. MS/MS analysis indicated that *in vitro*, CBP acetylates PCNA at residues Lys 13,14,77 and 80. Expression of PCNA mutated in these residues, resulted in impaired DNA replication and repair, enhanced sensitivity to UV radiation, and activated a DNA damage response. The same mutations prevented removal of chromatin-bound PCNA, and its subsequent proteolytic degradation after DNA damage. Depletion of CBP/p300 in normal fibroblasts prevented PCNA acetylation and degradation, while proteasome inhibition resulted in accumulation of acetylated PCNA. Our results suggest that PCNA acetylation by CBP and p300 is required to link DNA repair synthesis with chromatin removal and degradation of PCNA after DNA damage, to avoid an excessive accumulation of PCNA, dangerous for genome stability.

6 - Development, Differentiation and Aging

P6.1
Generation and characterization of a novel zebrafish reporter line for cAMP/CREB signaling

G. Busolin[1], O. Ek[1,2], P. Porazzi[1], A. Vettori[1], F. Marelli[3], M. Campione[4], L. Persani[3], F. Argenton[1], N. Tiso[1]
[1]Dept of Biology, Univ. of Padua, Padua, Italy, [2]Unit of Molecular Biology and Genetic Engineering, Univ. of Liège, GIGA-R, Liège, Belgium, [3]Dept of Clinical Sciences and Community Health, Univ. of Milan, Milan, Italy, [4]CNR Institute of Neuroscience, Padua, Italy

Cyclic AMP response element (CRE)-binding protein (CREB) is an important transcription factor that activates target genes in response to several stimuli, including peptide hormones, growth factors, and neuronal activity. CREB activation is induced through phosphorylation mediated by protein kinases, including protein kinase A (PKA), mitogen-activated protein kinases (MAPKs), and Ca^{2+}/calmodulin-dependent protein kinases (CaMKs). To investigate cAMP/CREB signaling pathway *in vivo*, we have generated transgenic zebrafish lines expressing *in vivo* GFP or mCherry under the control of a multimerized cAMP/CREB responsive element. At first, we observed that the CREB reporter is maternally activated and then detected in several embryonic tissues, including thyroid, muscles and cardiovascular system. Interestingly, cAMP/CREB signaling persists during animal adulthood, principally in fin joints and cardiac ventricle. To validate the specificity of these reporter lines, we applied both pharmacological and genetic approaches. Our findings demonstrate that CREB zebrafish reporter lines are sensitive biosensors able to visualize *in vivo*, in an intact organism, cAMP/CREB signaling pathway activity.

P6.2
Translational regulation in Drosophila spermatogenesis: the role of the DEAD-box RNA helicase Belle

N. Formicola, F. A. Digilio
Institute of Biosciences an BioResources CNR, Naples

Differential gene expression underlies the morphological diversity of differentiated cell types, and depends on regulation at both transcriptional and post-transcriptional levels. In D. melanogaster, most of the testes specific genes are transcribed predominantly pre-meiotically in primary spermatocytes. The mRNAs are stored in translationally inactive RNPs for translation during spermiogenesis. Several evidences suggest that distinct mechanisms of translational repression and activation act on specific mRNAs at different steps in the process. A key regulatory point for translational control is initiation, promoted by binding of the translational initiation complex to the 5' cap of the mRNA. Belle is a DEAD-box RNA helicase that is thought to be involved in many aspects of RNA metabolism, including pre-mRNA splicing, nuclear RNA export, RNA interference, translational repression and translational initiation. It has been reported that its yeast ortholog Ded1 directly assemblies a translational pre-initiation complex which represses translation. The role of Belle in spermatogenesis remains poorly understood, although the existence of a viable bel male-sterile mutant. To understand the role of belle in male germline development, we are characterizing this mutant line in order to identify potential interacting partners.

P6.3
Lack of FCD-2 protein during development induces developmental defects in *Caenorhabditis elegans*

M. Germoglio[1,2], A. Adamo[1], A. La Volpe[1]
[1]Institute of Biosciences and Bioresources, CNR, Napoli, Italy, [2]Second University of Naples, Caserta, Italy

Mutations in the *C. elegans fcd-2* gene induce genome instability, hypersensitivity to inter-strand cross-linking (ICL) agents and a significant increase in developmental defects. This gene is involved in double-strand breaks repair using homologous recombination and in preventing the careless repair by non-homologous-end-joining (NEHJ; Adamo *et al.*2010). Eliminating LIG-4, a key factor of NEHJ pathway, is sufficient to suppress the ICL hypersensitivity and developmental defects frequency of *fcd-2* mutants. Developmental defects in *fcd-2* mutants can be caused by meiotic/somatic mutations or alteration of the cell lineage. In order to understand the origin of developmental defects, we used a strain where the *fcd*-2 gene is maintained in heterozygosis with the nT1[unc-?(n754),let-?,qls-50](IV;V) balancer. The screening of the *fcd-2*first generation homozygotes shows a significant increase of developmental defects, due to lack of FCD-2 during development. In order to understand the mechanisms underlying the genesis of developmental defects in *soma,* we analyzed the early embryogenesis. The treatment with the ICL inducing agent cisplatin leads to a significant delay in cell division in wild-type and a greater increase in *fcd-2* mutants. This time-lag decreases in *fcd-2;lig-4* double mutants. This result suggests that activation of NEHJ can induce cell division delay likely associated with developmental defects.

P6.4
Effects of natural compounds present in *Chelidonium majus* on stem cells and embryo development

M.E. Isolani[1], A.M. Bianucci[2], L. Balestrini[3], A. Di Donfrancesco[3], P. Deri[3], D. Martini[3], M. Andreazzoli[3,4], R. Batistoni[3,4]
[1]Dept. Pharmacy, Pisa Univ. Italy; [2]Istituto Nazionale per la Scienza e Tecnologia dei Materiali, Florence, Italy; [3]Dept. Biology, Pisa Univ. Italy;[4]Centro Interdipartimentale di Ricerca Nutraceutica e Alimentazione per la Salute, Pisa Univ. Italy

Despite their potential attractiveness as model for regeneration, planarians have not been used yet in large-scale chemical screenings to test the effects of compounds on different key aspects of regeneration, such as stem cell proliferation and differentiation. Our work focuses on the analysis of the effects produced by the main alkaloids (chelidonine, berberine, sanguinarine) present in *Chelidonium majus*, an herb with therapeutical properties. The data obtained so far indicate that chelidonine generates anti-proliferative effects on planarian stem cells, possibly due to inhibition of tubulin polymerization, berberine induces a perturbation of the regenerative pattern and sanguinarine produces abnormal head regeneration. In particular, we find that berberine causes abnormal regeneration of the visual system, without affecting cell proliferation/apoptosis, while sanguinarine induces apoptosis through a caspase-dependent mechanism. These results clearly indicate the potentiality of planarians as a model to analyze drug effects. We are currently exploiting the well-established zebrafish model to assess and compare the effects of the same compounds during vertebrate embryo development.

P6.5
Impact of forced inclusion or exclusion of Extra Domain A (EIIIA) exon of fibronectin on adult murine hemopoiesis

A. Malara[1,2], C. Gruppi[1], L. De Marco[3], A. F. Muro[4], A. Balduini[1,2,5]
[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Laboratory of Biotechnology, IRCCS San Matteo Foundation, Pavia, Italy, [3]Department of Translational Research, National Cancer Center (IRCCS CRO), Aviano, Italy, [4]The International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, [5]Department of Biomedical Engineering, Tufts University, Medford, MA, USA

The expression and function of fibronectin (FN) containing the Extra Domain-A (EIIIA) in adult hemopoiesis is still unknown. Here we performed an *in vivo* study of the hemopoietic compartment in mice including ($EIIIA^{+/+}$) or excluding ($EIIIA^{-/-}$) the EIIIA exon. $EIIIA^{+/+}$ and $EIIIA^{-/-}$ mice showed reduced percentages of myeloid cells and immature B cells in bone marrow (BM), while the frequencies of hemopoietic stem and progenitor cells were comparable to wild-type mice. Moreover, only $EIIIA^{+/+}$ mice presented increased numbers of highly polyploid

megakaryocytes in BM, while platelet release *in vitro,* as well as *in vivo* after induced thrombocytopenia, was normal. Most importantly, after 5-fluorouracil induced myelosuppression, EIIIA segment interacted with Toll Like Receptor 4 (TLR-4) modulating cytokines and pro-fibrotic gene expression. Absence of EIIIA exon in the EIIIA$^{-/-}$ mice seemed to interfere with the EIIIA-TLR-4 axis resulting in a delayed BM recovery, while its inclusion improved survival outcome. In conclusion, FN EIIIA+ is not a critical regulator of steady state hemopoiesis, but its inclusion accelerates BM recovery and increases survival rate after myelosuppression.

P6.6

Potential involvement of *pdzrn3* gene during kidney morphogenesis

S. Marracci, A. Vangelisti, V. Raffa, M. Andreazzoli, L. Dente

Dept. of Biology, University of Pisa, SS12 Abetone e Brennero 4, PISA

Pdzrn3 is a multidomain protein, which has been implicated in myoblast and osteoblast differentiation and more recently in retinal ganglion cell development. We investigated *pdzrn3*spatio-temporal expression pattern in *Xenopus laevis* embryos. Dynamic *pdzrn3* expression was observed since neurula stages and later in several developing tissues, such as glomus, tubules and ducts of the pronephros, the *Xenopus* embryonic kidney very similar to the most primitive nephric structures of human kidney. To investigate the potential role of *pdzrn3* gene in pronephros morphogenesis, we performed loss-of-function experiments. We injected a specific *pdzrn3* antisense morpholino into one blastomere of two-cell embryos, at marginal zone level. Exploiting a set of pronephric markers, we could distinguish mild and severe phenotypes, showing deformation and enlargement of the proximal tubules, but not duct perturbations. Rescue experiments, in which mRNA of the orthologous zebrafish*pdzrn3* gene was injected together with the morpholino, allowed recovery of wild type phenotypes, confirming the pdzrn3 importance for a correct kidney morphogenesis.

P6.7

The transcription factor Nfix regulates the proper timing of post-natal muscle growth and regeneration

G. Rossi[1], S. Antonini[1], C. Bonfanti[1], S. Monteverde[1], C. Vezzali[1], S. Tajbakhsh[2], G. Cossu[3], G.Messina[1]

[1]Department of Biosciences, University of Milan, Via Celoria 26, 20133, Milan, Italy, [2]Stem Cells and Development, CNRS URA 2578, Department of Developmental & Stem Cell Biology, Institut Pasteur, 25 rue du Dr. Roux, 75015 Paris, France, [3]Department of Cell and Developmental Biology, University College London, Rockefeller Bldg. 21 University Street, London WC1E 6BT; Department of Biolosciences, University of Milan, 20129, Milan, Italy

Nfix is part of a family of highly conserved proteins that act as transcriptional activators and/or repressors of cellular and viral genes. Recently, Nfix has been found to be able to drive the transcriptional switch between embryonic and fetal myogenesis (Messina et al, 2010). During post- natal life, *nfix* null mice have a reduced body mass and are unable to fully extend their limbs (Driller et al, 2007), although no published information is still available about Nfix function in adult skeletal musculature. We therefore took advantage of an *nfix* null mouse model to study the involvement of this transcription factor in post-natal myogenesis. Interestingly, in the absence of Nfix we found an alteration of the muscle phenotype, with a reduced cross sectional area of the myofibers and a conversion toward a slower twitching phenotype. Moreover, we demonstrate that Nfix is necessary for the proper timing of satellite cell differentiation and muscle regeneration after injury, and unravel that Nfix exerts its function through a direct repression of the myostatin promoter. Overall the results obtained prompted us to propose that Nfix is necessary for the proper orchestration of post-natal myogenesis and for the correct timing of regeneration after damage.

P6.8

P63, a new molecular target of the teratogenic drug Thalidomide

E. Molinari, T. Lopardo, S. Cermenati, M. Restelli, A.Tramontini, M. Beltrame, L. Guerrini

Dept. of Biosciences, University of Milan, Milano, Italy

In the 1950s, severe developmental malformations were detected in babies born to mothers exposed to the drug Thalidomide (Th). Th is now used in the treatment of leprosy and multiple myeloma but little is known about its mechanism of action. The transcription factor p63 plays a key role in limb, epithelial and cranio-facial development and p63 mutations are associated to human congenital syndromes. In some cases, the clinical features of p63 patients were similar to Th-induced malformations. This observation prompted us to investigate whether p63 could be a molecular target of Th. Our data indicate that ΔNp63α and ΔNp63β proteins are degraded through the proteasome upon Th exposure in cell lines expressing either the endogenous or the transfected p63 proteins. We identified p63 residues serine 383 and threonine 397 as the aminoacids required for Th-induced p63 degradation. Th promotes p63 degradation not only in cell lines but also *in vivo*; when treated with Th, zebrafish embryos show a decrease in p63 protein levels along with a reduction in the length of pectoral fins, which is reverted by the overexpression of p63. The results shed new light on the molecular mechanisms of Th.

P6.9

FGF8, c-Abl and p300 cooperate in the regulation of ΔNp63α protein stability

M. Restelli[1], B. Marinari[2], D. Conte[3], N. Gnesutta[1], E. Molinari[1], A.V. Tramontini[1], A. Costanzo[2], G.R. Merlo[3], L. Guerrini[1]

[1]Dept. of Biosciences, University of Milan, Milan Italy, [2]Dept. of Dermatology, University of Rome Tor Vergata, Rome, Italy, [3]Dip. Biotecnologie Molecolari Scienze per la Salute 10126 Torino, Italy

The p63 transcription factor, homolog to the p53 tumor suppressor, plays a crucial role in epidermal and limb development. Dominant mutations in the *p63* gene give rise to several human congenital syndromes characterized by skin, craniofacial and limb defects. However, little is known on the post-translational modifications controlling ΔNp63α functions. Here we report that, FGF8 is an upstream signal inducing ΔNp63α stabilization and activation in human cells and in mice embryonic limb buds, *ex vivo*. FGF8 determined an increase in the binding of ΔNp63α with the tyrosine kinase c-Abl and in the level of ΔNp63α acetylation. We identified p300 as the acetyl-transferase catalizing acetylation on lysine 193 that triggers ΔNp63α stabilization and transcriptional activation upon FGF8 treatment. Our results link together FGF8, c-Abl, p300 in a regulatory pathway essential for the activation and stabilization of ΔNp63α: mutations or altered expression of components in this pathway are associated to abnormal limb development and might contribute to the molecular pathogenesis of Split Hand/ Split Foot Malformation.

P6.10

RNA-seq and Insect pests: new methods for the identification and the analysis of sex-specific genes in species of economic or sanitary interests

V. Petrella, A. Meccariello, G. Saccone, M. Salvemini

Department of Biology, University of Naples Federico II, Naples, Italy

Insects are among the largest taxonomic animal groups on Earth. In some cases, their interactions with humans can be harmful and the knowledge of the lifecycle of insect species, e.g. disease vectors and agricultural pests, opens new chances to develop eco-sustainable control strategies, alternative to pesticides. Reproduction and sex determination are at the same time crucial aspects to understand insect biology and optimal targets to limit their population growth and diffusion. We are applying RNA-seq and digital gene expression analysis to the study of sex determination in Insect species. In particular we are comparing *de novo* assembled transcriptomes of early sexed embryonic populations of

the agricultural pest *Ceratitis capitata*(Diptera, Tephritidae) to identify the unknown primary signal of sex determination in this species. We are producing de novo transcriptomes fromadult males and females of the two hematophagous dipteran non-model species *Aedes albopictus* (Diptera, Culicidae) and *Phlebotomus perniciosus*(Diptera, Psychodidae), to identify genes with sex-specific or sex-biased expression, potentially involved in sex determination pathway of these vector species. We are also developing new graphical interfaces and on-line databases for comparative genomic analyses and digital gene expression analyses in biological samples with opposite sexes.

P6.11
microRNA-494 promotes cellular senescence in Human Diploid Fibroblasts by targeting several genes

M. Succoio[1,3], M. Comegna[1,3], M. Napolitano[2], M. Vitale[1,3],C. D'Ambrosio[4], A. Scaloni[4], F. Passaro[1,2,3], N. Zambrano[1,3], F. Cimino[1,2,3], R. Faraonio[1,3]

[1]Dipartimento Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Napoli, Italy, [2]Fondazione SDN – Istituto di Ricerca Diagnostica e Nucleare, Napoli, Italy, [3]CEINGE-Biotecnologie Avanzate s.c. a r.l, Napoli, Italy, [4]Proteomics and Mass Spectrometry Laboratory, National Research Council, Napoli, Italy

We recently identified miR-494 up-regulation as a component of the program leading to cellular senescence of human diploid IMR90 fibroblasts [1]. To identify miR-494 targets, we used 2D-DIGE coupled to mass spectrometry analysis to profile protein changes induced by overexpression of miR-494 in IMR90 cells. miR-494 induced robust perturbation of the IMR90 proteome by significantly ($p \leq 0.05$) affecting a number of proteins. Combination of mass spectrometry-based identification of down-regulated proteins and bioinformatic prediction analysis for miR-494 binding sites on the relevant mRNAs, identified 26 putative targets of miR-494, with 7 of them featuring evolutionary conservation of miR-494 binding site. Functional miR-494 binding site were confirmed for hnRNPA3, PDIA3, RAD23B, and SYNCRIP. siRNA-mediated knockdown of hnRNPA3 and, to a lesser extent, RAD23B mirrored the senescent phenotype induced by miR-494, blunting cell proliferation and causing increase of SA-β-gal and DNA damage. Reintroduction of hnRNPA3 or RAD23B slowed the appearance of miR-494-induced senescent phenotype in IMR90 cells. Overall, these findings identify novel miR-494 direct targets involved in senescence.

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P6.12
Nerve Growth Factor (NGF) and its receptor level correlates with Osteoarthritis progression: implication with Matrix Metalloproteinase secretion in synovial fluid

R. Tiribuzi[1], C. Montagnoli[1], L. Crispoltoni[2], A.M. Stabile[2], A. Pistilli[2], M. Rende[2], G. Cerulli[3]

[1]Istituto di Ricerca Traslazionale per l'Apparato Locomotore Nicola Cerulli-LPMRI, Biology and Regenerative Medicine Division, Arezzo, Italy;, [2]Dipartimento Scienze Chirurgiche e Biomediche, Università degli Studi di Perugia, Italy;, [3]Istituto di Clinica Ortopedica e Traumatologica, Università Cattolica del Sacro Cuore-Policlinico Universitario Agostino Gemelli, Roma, Italy;

NGF and its receptors TrKA and p75 play a crucial role in the inflammatory process. Osteoarthritis (OA) is a degenerative joint disease associated with aging, characterized by low grade inflammatory process and cartilage degeneration mediated primarily by MMPs. Previous studies have shown the involvement of NGF signalling in OA, but its role is still unclear. The aim of this study was to investigate the NGF amount and the expression level of its receptors on cells isolated from synovial fluid (SF) taken from OA patients who underwent total knee arthroplasty (TKA). Our results show that the amount of soluble NGF and the expression of its inducible receptor (p75), and inflammatory monocyte ($CD14^{+}/CD16^{++}$) levels increases in OA patients and correlates with OA progression. Moreover, *in vitro* studies show that the expression of p75 and MMP2 were increased in NGF-treated-monocytes, suggesting a potential role in OA progression. In conclusion, our data show that, beside the release of NGF, there is a significant involvement of p75 receptor in the innate immune response during OA progression, hence p75 could represent a new therapeutic tool in OA treatment.

O6.1
Megakaryocytes express extracellular matrix components

V. Abbonante[1,2], C. A. Di Buduo[1], C. Gruppi[1,2], A. Malara[1,2], U. Gianelli[3], G. Barosi[1,2], V. Rosti[1,2], A. Balduini[1,2,4]

[1]Dept of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Laboratory of Biotechnology, IRCCS San Matteo Foundation, Pavia, Italy, [3]Dept of Pathophysiology and Transplantation, Division of Pathology, Hematopathology Service, Univ. of Milan Medical School, IRCCS Ca' Granda - Maggiore Policlinico Hospital Foundation, [4]Dept of Biomedical Engineering, Tufts University, Medford, MA, USA

Bone marrow extracellular matrix (ECM) components play a fundamental role in the regulation of megakryocyte (Mk) development. In this work we showed that human Mks synthesize their own various ECM components and we found a direct dependency between the concentration of thrombopoietin (TPO), the main regulator of megakaryopoiesis, and this ECM component synthesis, *in vitro*and *in vivo*. The mechanism responsible appeared to be the increased TGF-b1 release by Mks and consequent downstream pathways activation. Consistently, we also showed that patients with primary myelofibrosis present higher concentration of plasma TPO and a thicker peri-cellular matrix around Mks correlating with the grade of bone marrow fibrosis. Finally, i*n vitro* differentiated Mks from the same PMF patients showed up-regulation of ECM component synthesis that was restored to normal level after treatment with the inhibitor of the TPOR downstream mediator, JAK. Concluding we demonstrated, for the first time, that human Mks are ECM components producing cells, and we shed light on the mechanisms that regulate this process in physiologic and pathologic conditions, both *in vivo* and *in vitro*.

O6.2
Unacylated ghrelin promotes skeletal muscle regeneration

S. Reano, E. Angelino, O. Sabry, M. Ferrara, A. Graziani, N. Filigheddu

Department of Translational Medicine, Università del Piemonte Orientale "Amedeo Avogadro", Novara, Italy

Acylated and unacylated ghrelin (AG and UnAG, respectively) are hormones products by the Ghrl gene. AG, but not UnAG, stimulates growth hormone release, appetite, and positive energy balance through binding to its receptor GHSR1a, whereas both peptides act directly on skeletal muscles promoting differentiation of C2C12 myoblasts and preventing skeletal muscle atrophy without the involvement of GHSR1a. Based on these findings, we aimed to investigate if UnAG affected skeletal muscle regeneration by exploiting Myh6/Ghrl transgenic mice (Tg), characterized by constitutively high UnAG circulating levels. Skeletal muscle regeneration was improved in Tg mice, with new-formed myofibers characterized by increased area and number of central nuclei. Moreover, the greater number of quiescent Pax7+ve satellite cells (SC) in Tg muscles compared to WT suggest that a UnAG-rich environment may enhance SC proliferation and/or self-renewal. Finally, the expression of Ghrl gene, barely detectable in skeletal muscle, is strongly induced during regeneration. These findings suggest that AG/UnAG may act as novel myokines playing a central role in skeletal muscle stress-induced adaptive response.

O6.3
Silencing of Drosophila H/ACA snoRNP pseudouridine synthase dysregulates key developmental pathways

L. F. Iannucci, A. Di Giovanni, A. Petrizzo, C. Romano, R. Vicidomini,

M. Furia
Department of Biology, University of Naples "Federico II", Complesso Universitario di Monte Sant'Angelo, via Cinthia I, 80126

The Drosophila *mfl/Nop60b* gene belongs to a highly conserved family of ubiquitous nucleolar proteins that act as pseudouridine synthases and are involved in ribosome biogenesis and pseudouridylation of RNAs. Hypomorphic mutations of the human ortholog, the *h-DKC1* gene, cause the X-linked Dyskeratosis Congenita (X-DC) disease, whose main symptoms are bone marrow failure, stem cell deficiency, telomere shortening and increased tumor susceptibility. In order to define in detail the effects triggered by pseudouridine synthase loss of function, we induced *mfl* gene silencing *in vivo* within specific regions of the Drosophila wing disc. Intriguingly, we found that upon gene silencing the rate of protein synthesis was not generally reduced, while the activity of key developmental pathways was instead strongly dysregulated. *mfl*silencing causes in fact a strong up-regulation of *Notch*activity at the D/V boundary, while JAK-STAT and JNK pathways are ectopically induced at specific regions of the silenced wing disc. These results indicate that *mfl/Nop60b* gene can specifically regulate the activity of key developmental pathways, linking growth and proliferation to differentiative events.

O6.4
Epigenetic regulation of positional identity in neural progenitors

N. Carucci, V. Licursi, E. Cacci, C. Soldati, G. Augusti-Tocco, S. Biagioni, R. Negri, G. Lupo
Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, Rome, Italy

During development, neural progenitor cells (NPCs) acquire different anteroposterior (AP) positional identities under the influence of morphogens such as Retinoic Acid (RA). Following this initial specification, NPCs stably maintain their positional identities, while modifying their competence to respond to morphogen signaling. The molecular mechanisms controlling maintenance of positional identity and morphogen response in NPCs, however, remain poorly understood. We derived NPCs from the rostral (cerebral cortex, Ctx) and the caudal region (spinal cord, SC) of mouse embryos after AP patterning has taken place. Ctx and SC NPCs maintained in vitro expression profiles consistent with their region of derivation in vivo. For example, SC NPCs expressed Hoxb genes, while Ctx NPCs did not. We challenged these expression patterns by exposing NPCs to RA. Even though the RA pathway was functional in both Ctx and SC NPCs, RA treatments increased Hoxb expression in SC NPCs, but not in Ctx NSCs. We then analysed deposition of specific histone marks at the level of selected regulatory regions in Hoxb genes, finding significant differences in the epigenetic signature of the Hoxb genetic region in Ctx vs SC NPCs. These results suggest that differential epigenetic modifications maintain Hox responsiveness to RA in SC NPCs and abrogate it in Ctx NPCs. We are carrying out additional work to clarify the interactions between epigenetic modifications and RA-dependent Hox gene regulation in NPCs.

O6.5
Hmga2 is required for the neural crest cell EMT and migration in the *Xenopus* embryo

S. Macrì[1,2], L. Simula[1,2], I. Pellarin[3], M. Onorati[1,2], S. Pegoraro[3], R. Sgarra[3], G. Manfioletti[3], R. Vignali[1]
[1]Dipartimento di Biologia, Unità di Biologia Cellulare e dello Sviluppo, SS12 Abetone e Brennero 4, 56127 Pisa, Italy, [2]Istituto Toscano Tumori, via T.Alderotti 26/N, 50139 Firenze, Italy, [3]Dipartimento di Scienze della Vita, via Giorgieri 5, 34127 Trieste, Italy

HMGA factors are small proteins that bind DNA by "AT-hook" motifs, modify chromatin and assist in gene expression. Two genes, *Hmga1* and *Hmga2*, are present in mammals. They are highly expressed during embryogenesis, or reactivated in human tumors, where they play a role in the epithelial-mesenchymal transition (EMT). We studied the developmental role of*Xenopus* Hmga2 (Xhmga2) in the EMT of neural crest cells (NCCs), a cell population that migrates to diverse regions of the embryo to produce several differentiated tissues. Interfering with Hmga2 function by morpholino injections leads to severe disruption of the major skeletal derivatives of cranial NCCs. By loss- and gain-of function approaches, molecular marker analysis and NCC transplantation experiments, we determined the position of *Xhmga2* in the NCC genetic program and found that it is required for the EMT of NCCs and their migration to the branchial pouches. Next, we compared the effect of siRNA interfering on HMGA2 function in a mammalian cellular model of EMT, showing similar (but not identical) effects on the genes assayed in *Xenopus* NCCs. Thus, the developmental role of Hmga2 in NCCs parallels its involvement in tumor EMT.

7 - Environmental Microbiology and Biotechnology

P7.1
Increased mitochondrial pyruvate dissimilation in yeast

G. Agrimi[1], M. C. Mena[1], K. Izumi[2], I. Pisano[1], L. Germinario[1], H. Fukuzaki[2], L. Palmieri[1], L. M. Blank[3], H. Kitagaki[2,4]

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Via Orabona 4, 70125 Bari, Italy, [2]Department of Environmental Sciences, Faculty of Agriculture, Saga University, 1 Honjo-cho, Saga 840- 8502, Japan, [3]Institute of Applied Microbiology - iAMB, ABBt – Aachen Biology and Biotechnology Department, RWTH Aachen University, 52074 Aachen, Germany, [4]Department of Biochemistry and Applied Biosciences, United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan

In yeast, pyruvate is placed at the crossroad of fermentation, oxidative metabolism and anabolic pathways. In this study we have characterized a previously developed pyruvate undersecreting sake yeast obtained by isolating a strain (TCR7) tolerant to ethyl α-transcyanocinnamate, an inhibitor of pyruvate transport into mitochondria. To obtain insights into pyruvate metabolism, we investigated the mitochondrial activity of TCR7 by oxigraphy and ^{13}C-metabolic flux analysis. The mutant strain (TCR7), displayed an higher mitochondrial pyruvate influx and oxidation, and a decreased glycerol production compared to the reference strain. These results indicate that mitochondrial activity is elevated in the TCR7 strain with the consequence of decreased pyruvate extracellular secretion. Surprisingly mitochondrial activity resulted much higher in the sake yeast compared to CEN.PK 113-7D, the reference strain employied in metabolic engineering. When shifted from aerobic to anaerobic conditions, sake yeast retained a branched mitochondrial structure for a longer time than laboratory strains. Further studies are needed to unveil the molecular mechanisms underlying these phenotypes.

P7.2
New technologies and tools for second generation bioethanol production: the BIOASSORT project

A. Amore[1], R. Liguori[1], B. Paramaswaren[2], V. Faraco[1]

[1]DeptChemical Sciences, University of Naples "Federico II", Italy, [2]Council Of Scientific and Industrial Research, Trivandrum, Kerala, India

Western Ghats regions (Kerala) biodiversity was explored for isolation of new microorganisms producing enzymes needed in lignocellulose conversion into fermentable sugars, namely cellulase and xylanase. 93 microorganisms were isolated and screened for these enzymes production. Particular attention was focused on the *Bacillus*sp. XR44A, producing the highest xylanase activity level. The enzyme responsible for this activity was identified through zymography and mass spectral analyses. The new xylanase acitivity was characterized from the catalytic point of view and showed to be a potential biocatalyst for the saccharification of pretreated Brewer's spent grain. These experiments were carried out within the BIOASSORT PROJECT ((318931)_Marie Curie Actions People, International Research Staff Exchange Scheme 2012, coordinator: Prof. Vincenza Faraco), aimed at the "Improvement of technologies and tools, e.g. biosystems and biocatalysts, for waste conversion to develop an assortment of high added value eco-friendly and cost-effective bio-products". Fuel ethanol is on of the main target products of the platform, together with lactic acid, polylactic acid and polyhydroxyalkanoates.

P7.3
Persistance and effect on the soil native community of a mycorrhizal fungi-based Inoculum in the rhizosphere of maize plants

A. Berruti, R. Borriello, E. Lumini, V. Bianciotto

Istituto per la Protezione Sostenibile delle Piante (IPSP) del CNR c/o Università di Torino, Torino, Italy

Arbuscular Mycorrhizal Fungi (AMF) are a group of root obligate biotrophs that exchange benefits with almost 90% of plant families. They provide the host with water, soil mineral nutrients and pathogen protection. In exchange, photosynthetic compounds are transferred to the fungus. Therefore, AMF are primary biotic soil components that, when missing or impoverished, e.g. due to anthropic input, can lead to a less efficient ecosystem functioning. Consequently, a process that aims at the re-establishment of the natural level of AMF richness is pivotal towards the restoration of the soil microbial balances. The main strategy to achieve this goal is the direct re-introduction of an AMF pool (inoculum) into the target soil. After inoculation, it is essential to verify whether the inoculum effectively has the potential to colonize the host plant, persist into the soil, and affect the native AMF community. In this study, we molecularly characterized and traced – through 454-pyrosequencing – the components of a commercial AMF-based inoculum applied to the maize rhizosphere in an arable field. The inoculum persistence and effects in shaping the soil native AMF community are discussed.

P7.4
Frequency of heavy metal and antibiotic resistance in enterococci isolated from coastal marine sediments

S. Pasquaroli[1], S. Giaconi[1], A. Di Cesare[1], C. Vignaroli[1], B. Citterio[2], F. Biavasco[1]

[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy, [2]Department of Biomolecular Sciences sect. Toxicological, Hygiene, and Environmental, Sciences, University of Urbino Carlo Bo - Urbino (PU), Italy

The spread of antibiotic (AB) resistance is a topical issue. Heavy metals (HM) in aquatic systems may contribute to co-selection of AB-resistant bacteria including enterococci, which are used as FIB and give rise to human infections. The frequency of HM and AB-resistant enterococci was assessed in sediment from three marine coastal areas [2 bathing sites (S, P) and a site near an oil refinery (FM)] in Marche, Italy. Susceptibility to copper (Cu), cadmium (Cd), mercury, ampicillin, chloramphenicol, ciprofloxacin, erythromycin (ERY), tetracycline (TET), streptomycin, vancomycin, quinupristin/dalfopristin and their resistance genes [*tcr*B, *cad*A, *blaZ*, *erm*A, *erm*B, *mef*, *tet*M, *tet*L, *tet*O, *ant*(6)-Ia] were determined. There were 123 strains (15S, 28P, 80FM); 84 (68%) were resistant to at least one HM (67%S, 79%P, 65%FM). *cad*A-carrying isolates were found at all sites; no *tcr*B-carrying strains were found in P. Fifty enterococci (41%) were resistant to at least one AB (20%S, 54%P, 40%FM); *erm*B and *tet*M were the most frequent resistance genes at all sites. In 38 (31%) isolates showed both HM and AB resistance (7%S, 50%P, 29%FM); the most frequent associations were Cd-TET (17%), Cd-ERY (15%) and Cu-TET (9%). Data indicate that sediment can be a reservoir of resistant enterococci and a potential public-health risk and suggest that the selective pressure exerted by the marine environment, including HM, can contribute to AB-resistant enterococci persistence.

P7.5
A world inside a glass of drinking water: community analysis and dynamics of antibiotic resistance genes

A. Bruno, A. Sandionigi, M. Barbuto, A. Galimberti, V. Mezzasalma, M. Casiraghi

Dept Biosciences and Biotechnologies, Milano-Bicocca Univ., Milan, Italy

Water is a complex matrix in which microorganismsinteract in a dynamic network.Quality of drinking water is a public health concern worldwide. Recent studies demonstrated thatdrinking water treatment process can affect the microbiome structure.Moreover the occurrence and spread of antibiotic resistance genes (ARGs) in water is becoming an issue ofgreat interest. A multilevel approach can open new perspectives in environmental researches. Molecular techniques can give a deeper

knowledge, going beyond the limit of culture-dependent methods. We collected water samples in a water treatment plant in Milan (Italy), at different steps of treatment process, from the source to the tap.In this pilot study we analyzed the presence and the relative abundance of several ARGs.Our preliminary results show a differential distribution of ARGs across the water treatment plant: disinfection selects for resistant bacteria and bacterial populations may exhibit differential resistance to the treatment. This analysisis integrated in a broader study characterizing microbiome structure variability using High-Throughput Sequencing, in order to better understand this complex ecosystem.

P7.6
Bi-fuel supply is adopted by *Rhodobacter sphaeroides* to cope with high concentrations of Cobalt ions

M. Volpicella[1], A. Costanza[1], O. Palumbo[2], F. Italiano[3], C. Leoni[1], A. Placido[4], E. Picardi[1], M. Carella[2], M. Trotta[3], L.R. Ceci[4]
[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy [2]Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG), Italy [3]Institute for Chemical-Physical Processes, CNR, Bari, Italy [4]Institute for Biomembranes and Bioenergetics, CNR, Bari, Italy

Rhodobacter sphaeroides is known for its adaptive capacities to different environmental and nutritional conditions, including presence of heavy metals. It is therefore a valuable model organism for understanding bacterial adaptation to metals and possible applications for bioremediation of polluted sites. To characterize the capability of *R. sphaeroides* to cope with high cobalt ion concentrations, we combined selection of adaptive defective mutants, carried out by negative selection of transposon insertional libraries on 5 mM Co^{2+} plate, with analysis of growing capacities and transcriptome profiling. Comparative analysis of results from a selected mutant and wild-type strains indicated that the adaptive ability of *R. sphaeroides* strongly relies on its ability to exploit any available energy-supplying metabolisms, being able to behave as photo- or chemotrophic microorganism. The selected mutant exhibited a severe down-expression of a sugar ABC-transporter, which results non-permissive for its growth in cobalt-enriched media under aerobic and no-photosynthetic conditions. Interestingly, the defective expression of the transporter does not prevent the mutant growth under photosynthetic conditions.

P7.7
Conversion of the genotoxin 4-nitroquinoline-1-oxide into inactive products by a probiotic *Lactobacillus rhamnosus* strain

A. Bocci, B. Sebastiani, F. Trotta, E. Federici, G. Cenci
Dept Chemistry, Biology & Biotechnology, Perugia Univ., Italy

4-nitroquinoline-1-oxide (4-NQO) is a nitroarene with high genotoxicity. Our previous studies showed the ability of some microorganisms to counteract its biological activity. This finding, confirmed by different short-term methods (SOS-Chromotest and Comet assay), specified that the loss of genotoxicity observed in cell-free supernatants was not related to 4-NQO adsorption. There are little information about the chemical-biological interactions. We investigated the mechanisms of 4-NQO inhibition by a probiotic *Lactobacillus rhamnosus* strain (IMC501) characterized by murine model regarding protective effect against DNA damage induced by food mutagens. Inhibition was associated with hypsochromic shift of UV-profile with a progressive absorbance increase of the new peak. GC/MS and IR-Raman analyses confirmed (a) the disappearance of 4-NQO, (b) the absence of any potentially genotoxic metabolites (*i.e.* 4-HAQO and 4-NOQO) and (c) the presence of phenylquinoline, and related isomers, as not genotoxic bioconversion products. These findings, beside interest as basic knowledge, can contribute to the further definition of non-conventional functional properties of probiotics.

P7.8
Genomic and phenotypical characterization of the electroactive bacteria *Enterobacter*sp. EAN3: preliminary results

C. Chiellini[1], I. Palchetti[2], F. Bettazzi[2], D. Voccia[2], M. Migliore[3], M. Fondi[4], F. Canganella[5], E. Di Mattia[6], R. Fani[4], A. Benedetti[3], S. Mocali[1]
[1]Consiglio per la Ricerca e Sperimentazione in Agricoltura - Centro di Ricerca per l'Agrobiologia e la Pedologia (CRA-ABP), P.za D'Azeglio, 30, 50121 - Florence, Italy, [2]Dipartimento di Chimica, Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy., [3]CRA—Research Centre for the Soil–Plant System, via della Navicella 2, 00184 Roma, Italy, [4]Department of Biology, University of Florence, Via Madonna del Piano 6, -50019 Sesto Fiorentino (Florence), Italy, [5] Department for Innovation in Biological, Agrofood and Forest systems, University of Tuscia,via C. de Lellis,01100 Viterbo, Italy, [6]Department of Sciences and Technologies for Agriculture, Forest, Nature and Energy, University of Tuscia, via C. de Lellis,01100 Viterbo, Italy

The electroactive bacterium *Enterobacter*sp. EAN3 was previously isolated from a Microbial Fuel Cell inoculated with soil. The genome analysis and the phenotypic characterization of the strain by Phenotype Microarray (PM) were carried out to highlight the metabolic pathways involved in the electrogenic process. Current generation and biofilm development of EAN3 strain have also been observed by cyclic voltammetry and Scanning Electron Microscope respectively. The PM results from EAN3 strain were compared to the ones from Pseudomonas sp. CMR12a, a well known electroactive strain, and its mutant CMR- lacking the ability to produce electricity. Preliminary results highlighted that EAN3 could use a double mechanism for transferring electrons and 20 substrates in PM plates showed significant differences ($AV \geq 3$) in the metabolic activity between the electroactive strains (EAN3 and CMR12a) and the negative control, suggesting the existence of metabolic pathways peculiar of electricity generation. Moreover, some enzymes involved in the electron transfer chain, such as aldehyde dehydrogenase cystathionine-γ-lyase and cystathionine-γ-synthase were found to play a role in current generation.

P7.9
Phytoremediation by algae: a step of a multi-stage biotechnological process of bioremediation of an hydrocarbon sludge

A. Ciurli, A. Pardossi
Dept of Agriculture, Food and Environment Univ., Pisa, Italy

The project MARƎ aimed at developing a multi-stage treatment process of hydrocarbon sludge for the production of an gelling agent to be used in marine remediation of oil spills. Such product, obtained by fatty acids oxidation extracted by algae, will be capable of gelling oil spillage to facilitate and maximise its recovery. Our department worked only in second-stage of the process to achieve algae capable of growing on hydrocarbon sludge coming from first-stage bioremediation treatment. Growth parameters such as optical density, cell counting, content of photosynthetic pigments and biomass were estimated for six species of algae. Subsequently all the species were grown in their optimal growth medium but at increasing concentrations of sludge. From this first selection were identified two species and then they were acclimatized to grow at increasing concentrations of sludge until obtaining the complete survival of the algae to the sludge.

P7.10
Impact of desert dust deposition on the bacterial community of mountain glacier snow.

E. Montalbani, B. Moroni, R. Selvaggi, B. Sebastiani, M. Pecci, P. D'Aquila, G. Cenci, D. Cappelletti, E. Federici
Dept. of Chemistry, Biology and Biotechnology, University of Perugia, Italy

Dust particles in desert storms can move over great distances in the atmosphere and the uplifted microorganisms may survive to long-range transport. African desert storms cross the eastern Mediterranean and inject

a large pulse of microorganisms into the European air, expanding their biogeographical range. The impact in anthropized areas may be difficult to study because of the complexity of the urban scenario. Conversely, remote ecosystems, such as mountain glacier, are interesting case studies because more sensitive to exogenous microorganisms inputs. We have studied bacterial communities in snow samples from Calderone glacier (Gran Sasso d'Italia, 2700m asl) before and after desert dust deposition. DGGE and qPCR analyses indicated that different populations and amount of bacteria characterized these samples. Sequencing of 16S rRNA allowed identifying these populations, which included species known to be present in cold environments but also, in the dust-impacted snow, species typical of soil and/or desert environments, thus confirming the snow colonization. This information will be correlated with the chemical characteristics of the dust particles in the collected snow samples.

P7.11
Characterization and monitoring of a microbial community responsible of an innovative biological process for sulfide removal from industrial waste

C. Giordano[1], F. Spennati[2], A. Melone[1], G. Mori[3], C. Vannini[1]
[1]Dipartimento di Biologia, Università degli Studi di Pisa, Pisa, [2]Dipartimento di Ingegneria Civile ed Ambientale, Università degli Studi di Firenze, Firenze, Italy, [3]Cuiodepur S.p.A., 56029, San Romano, Pisa

An innovative biotrickling filter reactor for biological removal of sulfide from gaseous industrial waste streams has been developed and set up in the frame of the BIOSUR project (LIFE11 ENV/IT/075). This is realized thank to the development of a specialized sulfur-oxidizer biomass on the filters. The involved microbial community was selected in a side-reactor (volume of 450 l) by incremental addition of sulfides in selective conditions: low pH (2-4) and a concentration of sulfides of 2-4 mg/l. Such a specialized biomass was characterized and monitored by means of T-RFLP fingerprinting and clone-library construction of 16S rRNA coding genes. Preliminary data indicate that about 65% of screened clones are represented by bacteria whose 16S rRNA gene sequence show a similarity higher than 97% with that of members of the genus *Halothiobacillus*, well-known as Sulfur Oxidizing Bacteria (SOB). This result has been also confirmed by isolation in SOB selective medium. Obtained data have been comparatively analyzed in order to monitor microbial community evolution from the start-up throughout the end of the experiment.

P7.12
Essential oils: possible use to fight *Burkholderia cepacia* complex strains

I. Maida[1], E. Perrin[1], M. Fondi[1], A. Mengoni[1], A. R. Bilia[2], G. Flamini[2], G. Pesavento[3], A. Lo Nostro[3], C. Calonico[3], M. Barnabei[3], C. Chiellini[4], V. Maggini[5], E. Gallo[5], A. Vannacci[5], L. Gori[5], F. Firenzuoli[5], R. Fani[1]
[1]Department of Biology, University of Firenze, via Madonna del Piano, 6 I-50019 Sesto Fiorentino (Firenze), [2]Department of Chemistry Ugo Schiff, University of Florence, Via della Lastruccia 3-13, I-50019 Sesto Fiorentino, Florence, Italy, [3]Health Sciences Dpt., University of Florence, Viale G. B. Morgagni, 48, I- 50134 Firenze, [4]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Agrobiologia e Pedologia (CRA-ABP) Piazza D'Azeglio 30, I-50121 - Firenze (Italy), [5]Center for Integrative Medicine, Careggi University Hospital, University of Florence, Florence, Italy

Essential oils (EOs) can inhibit the growth of a wide range of microorganisms. Particularly interesting, is the possible use of EOs to treat the *Burkholderia cepacia* complex (Bcc) infections that are difficult to eradicate. In this work we have tested the ability of EOs extracted from six medicinal plants (*Eugenia caryophyllata*, *Origanum vulgare*, *Rosmarinus officinalis, Lavandula officinalis, Melaleuca alternifolia*and *Thymus vulgaris*) to inhibit the growth of 80 strains belonging to the 18 known Bcc species. Data obtained have shown that all the Bcc strains exhibited a high degree of sensitivity to each of the six EOs tested. The most effective EOs have been those extracted from *O. vulgare, T. vulgaris*and *E. caryophyllata*. The determination of Minimum Bactericidal Concentration has revealed that the three above mentioned EOs had a bactericidal activity also when diluted up to 0.125% (vol/vol) on almost all strains tested. Moreover, no mutant strain Bcc resistant to the six EOs has been detected in our large-scale screening. We have shown that EOs from medicinal plants might represent a source for new antibacterial formulations to fight Bcc infections.

P7.13
A rapid method to improve selection of cellular clones targeted by the CRISPR/Cas9 system

F. Niccheri, R. Pecori, S. G. Conticello
Core Research Laboratory, Istituto Toscano Tumori - AOU Careggi, Firenze, Italy

RNA-guided endonucleases based on the CRISPR/Cas9 system have recently emerged as a simple and efficient tool for genome editing. These endonucleases use a small guide RNA (sgRNA) to specifically cleave DNA targets. Here we report a selection method to increase the efficiency of the CRISPR/Cas9 system. The method is based on a chimeric construct in which an mCherry coding sequence is separated from an out-of-frame BlasticidinS Resistance gene (BSR) by the sgRNA-target sequence: the target will be cleaved, and error-prone DNA repair will bring the BSR in frame with the mCherry. Cotransfection of this construct with a CRISPR/Cas9 system targeted to a specific genomic locus will provide the cells bearing an active CRISPR/Cas9 complex with a temporary resistance to BlasticidinS, thus allowing for an efficient selection. We have tested this approach by targeting the Aicda gene in CH12 cells, a murine B cell lymphoma cell line in which Class Switch Recombination can be induced: 75% of the clones resulted targeted (42% homozygotes), compared to ~27% (4% homozygotes) obtained using the standard protocol.

P7.14
Screening of thiosemicarbazones as antimycotic drugs

C. Nolli[1], L. Giovati[2], T. Ciociola[2], F. Bisceglie[3], G. Pelosi[3], A.Buschini[1], W. Magliani[2], T. Lodi[1]
[1]Dept. Life Sciences, University of Parma, Parma, Italy, [2]Dept. Biomedical, Biotechnological and Traslational Sciences, University of Parma, Parma, Italy, [3]Dept. Chemistry, University of Parma, Parma, Italy

Fungal infections have become a problem of increasing importance over the past last decades, especially in patients with immunological impairment, due to HIV infections, organ transplantations or cancer therapy. The most frequently diagnosed fungal infections are caused by pathogens of the genera *Candida*, *Cryptococcus* and *Aspergillus*. Current treatments use a limited number of antifungal drugs and the increased resistance to these molecules is now a great problem in medicine. Is then important to select and develop new antifungal therapeutics., Thiosemicarbazones have received great interest because of their chemical and biological activities, such as antibacterial, antivirus, antiamoebic, and antitumor activities in human pharmacology. In order to test if an antifungal activity is associated to some of these molecules we screened 16 different thiosemicarbazone molecules against a panel of fungal pathogens. Two of these compounds showed interesting activity. Results will be here presented.

P7.15
TALEs for every season: targeted DNA damage and live visualisation of DNA

R. Pecori, C.C. Mattioli, G. Mattiuz, S.G. Conticello
Core Research Laboratory, Istituto Toscano Tumori

Transcription activator-like effectors (TALEs) are a novel class of modular dsDNA-binding proteins from Xanthomonas. Reordering of the various modules allows the design of TALEs specific for any target DNA. Based on this, a new generation of genome-engineering tools have been developed by fusing them with different moieties: with the catalytic

domain of the FokI nuclease for gene-targeting; with fluorescent proteins to visualise repetitive genomic sequences; with transcription factors to induce gene expression. Here we present the use of TALEs in three different applications. We have first used TALE to target DNA damage to specific target sites on a GFP reporter gene using AID/APOBECs. These molecules are cytidine deaminases physiologically acting on DNA. Starting from an inactive GFP reporter construct, we have used the TAL-AID or TAL-APOBEC1 to restore its activity through mutations at Cs. In parallel we are setting up a system to visualise dsDNA in live cells with chimeric TALEs, each fused with two non-fluorescent fragments of the YFP. When the pair of TALEs binds to targets in proximity to each other, the partial YFP fragments complement each other and become fluorescent.

P7.16

Mycobacterium smegmatis as model system to investigate cell wall components in nontuberculous Mycobacteria

G. Merola[1], F. Perrone[1], A. Cordone[2], M. Sacco[1]

[1]Seconda Università degli Studi di Napoli, Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche (DiSTABiF), [2]Dipartimento di Biologia, Università Federico II

Glycopeptidolipids (GPLs) are major components of the cell wall outer layers of several nontuberculous mycobacteria. These molecules have been implicated in many aspects of mycobacterial biology, including host-pathogen interactions, sliding motility and biofilm development. The GPLs structure is conserved among mycobacteria and is made by a common glycosylated lipopeptide core that is variably modified by O-methylation and O-acetylation. In the fast growing *Mycobacterium smegmatis*, all the genes necessary for GPL biosynthesis are clustered in a single region of 65 kb and most of them have been identified experimentally or by *in silico*prediction. Here we report the phenotypic characterization of a mutant strain of *Mycobacterium smegmatis*mc^{2}155, carrying a null mutation in the MSMEG_0394 gene located in the GPLs cluster. The gene was thought to be part of an operon constituted of three genes (MSMEG_0393, MSMEG_0394, MSMEG_0395) and our hypothesis has been confirmed by RT-PCR analysis. The *M. smegmatis* mc^{2}155 Δ0394strain shows strong differences compared to the wild type strain in colony morphology, biofilm formation, sliding motility and the complete absence of GPLs production.

P7.17

Antibacterial, antifungal and antitumor activity of *Mytilus galloprovincialis'* protamine-like

A.Basile[1], M. Turano[1], A. Murolo[1], F. Caccavale[1], F. Pollice[1], G. Speranza[1], S. Avagnano[1], D. Drongitis[1], L. Fucci[1], E. Notomista[1], J. Ausió[2], M. Piscopo[1]

[1]Dept of Biology, University of Naples Federico II, Italy, [2]Dept of Biochemistry & Microbiology, University of Victoria, British Columbia, Canada

We have studied the antibacterial, antifungal and antitumor activity of *Mytilus galloprovincialis'* protamine-like. Nine bacterial strains were employed. They included two Gram-positive and seven Gram-negative bacteria. The same clinically isolated (CI) bacterial strains were used to compare the sensitivity to these proteins. Antifungal tests were done on 3 strains of fungi including: 1 potentially pathogenic yeast (*Candida albicans* CI) and 2 filamentous phytopathogenic fungi *Botrytis cinerea* and *Rhyzoctonia solani. Mytilus galloprovincialis'* protamine-like inhibited all bacteria strains with different MBC values ranging from 7.8 to 250 µg/mL and resulted active also on some tested bacteria strains that are generally resistant to conventional antibiotics. They showed also a fungistatic effect on all the fungi tested, with MFCs ranging from 16 to 32 µg/mL and cytotoxic effects on RKO colon cancer cells starting from a concentration of protamine-like 15 µg/mL.

P7.18

Regulatory and metabolic proteins differentially expressed during NAI-107 production in *Microbispora*sp. ATCC-PTA 5024

G. Gallo[1], E. Palazzotto[1], T. Faddetta[1], A. Giardina[1], R. Alduina[1], G. Renzone[2], P. Monciardini[3], T. Weber[4], M. Sosio[3], R. Pozzi[4], E. Stegmann[4], A. Russo[1], F. Sangiorgi[1], A. Scaloni[2], A. M. Puglia[1]

[1]Dept STEBICEF, Palermo Univ, Palermo, Italy, [2]Dept ISPAAM, CNR, Napoli, Italy, [3]NAICONS Srl, Milano, Italy, [4]Dept IIMIM, Eberhard Karls Univ., Tübingen, Germany

Microbispora sp. ATCC-PTA-5024, the producer of lantibiotic NAI-107, is a poorly characterized actinomycete. High throughput techniques, like proteomics, may give insights on strain molecular physiology and biochemical capability and, above all, on metabolic pathways and regulatory mechanisms associated with antibiotic production. Thus *Microbispora* differential proteomic analyses were comparatively carried-out on wild type, null and super-producer strains by mean of 2-D-Differential Gel Electrophoresis and mass spectrometry procedures. This study reveals differential regulation of proteins involved in many cell processes and metabolic pathways associated with NAI-107 production on-set and maintenance. In particular, gene ontology clusterization shows that during NAI-107 production proteins involved in amino sugar, nitrogen, phosphate and sulphur metabolism, oxidative stress and antibiotic biosynthesis and resistance are up-regulated while proteins involved in glycolysis, amino acid and nucleotide metabolism are down-regulated. Thus, these data reveal a comprehensive set of differentially regulated proteins which may play a key role in NAI-107 production. Altogether this information may be used to rationally improve NAI-107 production by *Microbispora* fermentation optimization or by strain improvement by genetic engineering of targeted genes.

P7.19

In planta heterologous expression of thermophilic enzymes for the degradation of the plant cell wall

A. Rosa[1], N. Betterle[1], C. Crosatti[2], L. Cattivelli[2], R. Bassi[1]

[1]Dept of Biotechnology, University of Verona, Verona, Italy, [2]CRA-GPG, Fiorenzuola d'Arda, Piacenza, Italy

In order to improve the degradation of lignocellulosic biomass for the production of biofuels, a set of three cellulolitic enzymes isolated from the thermophilic bacteria *Thermotoga neapolitana* were expressed in the apoplast of *Arabidopsis thaliana* and *Hordeum vulgare*. The enzymes were selected in order to target the main polisaccharides of the cell wall: a β-glucosidase, an endo β-1,4 cellulase and an endo β-1,4 xylanase. The recombinant enzymes obtained from *A.thaliana* displayed a similar activity and resistance to high temperatures as the ones observed for the thermozymes of *Thermotoga neapolitana.* The immunolocalization assay was conducted in order to determine the localization of the proteins in the apoplast. Finally, the saccharification efficiency was evaluated on different types of substrates, convalidating the sinergistic activity of the cellulases in degrading the plant cell wall. As for the enzymes obtained from *H. vulgare,*low amounts of non functional proteins were obtained, underlining the need to optimize the expression of enzymes with regard to the expression platform.

P7.20

Testing PhyloH, a new phylogenetic approach on diversity analyses of bacterial communities

A. Sandionigi[1], S. Vicario[2], A. Bruno[1], B. Balech[3], E. Ferri[1], M. Casiraghi[1]

[1]BTBS, Università degli Studi di Milano-Bicocca, Piazza della Scienza, 2, 20126 Milano, Italy, [2]Consiglio Nazionale delle Ricerche, Istituto di Tecnologie Biomediche (ITB), via Giovanni Amendola, 122/D, 70126, Bari, Italy, [3]Consiglio Nazionale delle Ricerche, Istituto di Biomembrane e Bioenergetica (IBBE), via Giovanni Amendola, 165/A, 70126, Bari, Italy

One of the most difficult areas in ecology is analyzing the diversity and its partitioning. Particularly, microbial ecology studies generate

a large amount of interlinked data, which are complex to explore and interpret. To cope with this limitations, numerous bioinformatics tools have been developed. One of these tools is PhyloH (Vicario and Balech, 2013), a user-friendly web application which uses phylogenetic entropy as a measure to analyse changes across biological communities. The phylogenetic entropy is a generalization of Shannon entropy based on the fact that different observed categories are not all equally different from each others, having a similar structure that could be modeled using a phylogenetic tree (Jost 2007, Chao et al. 2010). In this contribution we tested PhyloH in various data set of microbiome studies. We provide an overview of its potential, showing that diversity partitioning data are presented via easily-explorable vectorial images. Our results prove that such approach is robust and is an effective alternative method to explore diversity.

P7.21
Isolation and characterization of a microbial consortium capable to deplete total petroleum hydrocarbon in dredged sediments

G. Siracusa, L. Mariotti, R. Lorenzi, S. Di Gregorio
Department of Biology, University of Pisa, Pisa, Italy

The present work is part of the project Bioresnova, financed by the Fondazione Pisa *that ai*ms to offer innovative solutions in the field of decontamination and recovery of dredged sediments. The work was related to the isolation and characterization of microbial candidates capable to degrade hydrocarbons constituting the main source of organic contamination of dredged sediments in urbanized area. Actually a microbial consortium, autochthonous to the dredged sediments of the Navicelli canal in Pisa has been isolated for the capacity to completely deplete gasoline in liquid culture. The microbial candidates composing the consortium were identified as bacterial and fungal strains. The bacterial candidates utilize the gasoline as the sole carbon source. A total of two out of five fungal strains are capable to completely deplete gasoline in liquid culture. The characterization of the microbial candidates composing the consortium of interest has the final goal the designing of a "minimal consortium" in terms of number of candidates to be exploited in bioaugmentation of dredged sediments in dedicated solid phase bioreactor.

P7.22
Chemical-physical and biological removal of oily compounds from slops originated from oil transportation by tankers

G. Mancini[1], A. Luciano[2], A. Volta[1,3], S. Santisi[3,4], S. Cappello[3]
[1]Dept of Industrial and Mechanical Engineering, University of Catania, Italy, [2]ENEA Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Rome, Italy, [3]Institute for Coastal Marine Environment (IAMC) – CNR U.O.S. of Messina, Italy, [4]Ph.D School in "Biology and Cellular Biotechnology" of University of Messina, Italy

Several oil products may be released into marine or coastal environments by ship drains and/or different kinds of accidents. Many of these fluids are very stable emulsions, which make chemical treatment difficult and the normal separation processes (gravity, flocculation, skimming) by themselves are frequently ineffective. The conventional methods for treatment of discharges are coagulation and chemical de-mulsification followed by secondary clarifications, using aggressive chemicals. The water phase from chemical treatment has to be further treated to meet today's effluent discharge standard. This can be done by granular activated carbon (GAC) filtration. When GAC is saturated in pollutant, it needs to be regenerated with costly thermal or chemical treatments. There are few works on the potential bio-regeneration of GAC saturated by salty mixtures of biodegradable and non-biodegradable compounds. Based on this, the aim of this research work was to investigate the feasibility of coupling physical, chemical and biological processes to optimize the treatment, lowering costs. Specifically this study focuses on the role of a microorganisms mixed culture on bioregeneration of GACs.

P7.23
Stimulation of hydrocarbons biodegradation in a marine sediment contaminated by crude oil with biogenic pollutant mobilizing agents and biosurfactants

G. Zanaroli, A. Rosato, M.S. Parisi, F. Fava
Dept of Civil, Chemical, Environmental and Materials Engineering (DICAM), University of Bologna

The aim of this work was to assess the effectiveness of biogenic pollutant mobilizing agents and surfactants in enhancing the anaerobic hydrocarbons biodegradation in slurry microcosms of a crude oil actual-contaminated marine sediment from Gela harbor. Two commercial products based on plant extracts (SC1000™ and Aircraft Cleaner™), hydroxypropyl-β-cyclodextrins (HPB-CD), randomly methylated β-cyclodextrins (RAMEB-CD), two commercial soy lecithins having different hydrophilic/lipophilic balances (Textrol F and Solec C), bile acids and the microbial surfactants rhamnolipids and sophorolipids were tested. Redox potential decreased from +50 mV to -90/-130 mV after 8 weeks in the not amended controls and to -150/-200 mV after 3 weeks in the surfactant-supplemented microcosms. Significant biodegradation of *n*-alkanes occurred during 30 weeks of incubation only in the presence of sophorolipids (16%), Textrol F (16%), RAMEB-CD (33%) and HPB-CD (22%), suggesting that they were either used as carbon sources and/or increased the bioavailability of organic substrates and hydrocarbons.
This work was supported by the EU through the project KILL SPILL (G.A. n. 312139)

O7.1
Understanding the role of EPSs in BSCs: study of the physiological response of the phototrophic community to EPS removal

A. Adessi[1], R. Cruz de Carvalho[2,3], F. Rossi[1], J. Marques da Silva[2,4], C. Branquinho[2,3], R. De Philippis[1]
[1]Dept of Agrifood Production and Environmental Sciences, Univ. of Florence, Italy, [2]Departamento de Biologia Vegetal, Faculdade de Ciências, Univ. de Lisboa, Portugal, [3]Centre for Environmental Biology (CBA), Lisbon, Portugal, [4]Centre for Biodiversity, Functional and Integrative Genomics (BioFIG), Lisbon, Portugal

Biological Soil Crusts (BSCs) are widely recognized as beneficial to soil fertility due to their contribution to soils stabilization and to the increase in their carbon, nitrogen and moisture content, mitigating desertification effects. Amongst the wide diversity of organisms that compose BSCs, cyanobacteria are the first to appear after disturbance events, being major extracellular polymeric substances (EPSs) producers. Besides representing a huge carbon source, EPSs give an important contribution to the behavior of the crusts. Using non-invasive techniques (IRGA and chlorophyll fluorescence) to analyze photosynthetic performance, we studied the ecophysiology of the phototrophic community in BSCs and its response to partial removal of the polymeric matrix. Preliminary results suggests that susceptibility to photoinhibition increases after EPS partial removal. Evaluating the role of the EPSs in the community is essential to further understand the equilibrium of those systems and how they can prove beneficial for soil stabilization., Acknowledgments: ESSEM COST Action ES1104.

O7.2
Exploring the genetic basis con competition in bacterial symbionts: the *acdS* gene in the rhizobium *Sinorhizobium meliloti*

A. Checcucci[1], M. Bazzicalupo[1], A. De Carlo[2], G. Emiliani[2], A. Mengoni[1]
[1]Department of Biology, University of Florence, Sesto F.no (Firenze), Italy, www.dblage.unifi.it, [2]Centro Nazionale Ricerche-IVALSA, Sesto F.no (Firenze), Italy

Mutualistic cooperation is one of the most fascinating issue in evolutionary biology and legume-rhizobia symbiosis can represent a

befitting model example.The strains of the same rhizobial species show different symbiotic performances and few studies only are addressing their genetic basis.One of the most intriguing gene is that encoding the enzyme 1-aminocyclopropane-1-carboxylate(ACC)deaminase (*acdS*) present in the dispensable genome of the model species *Sinorhizobium meliloti*. It is involved in the cleaving of plant-produced ACC,the precursor of the plant stress hormone ethylene. The role of *acdS*in the mutualistic behavior of *S.meliloti* strains is still obscure as well as the selective benefit conferred.To clarify this issue, an ortholog of *acdS* was cloned and expressed in the model strain *S.meliloti* Rm1021,that was then tested to evaluate its symbiotic phenotype with respect to competition for root nodule occupancy and modulation of ethylene production by the host plant. Data showed that *acdS* may provide a selective advantage,but a decrease of ethylene levels may reduce also the overall fitness of plants,then providing a balancing selection effect at the rhizobial population level.

O7.3
Domestication of the green alga *Chlorella sorokiniana*: reduction of antenna size improves light-use efficiency in a photobioreactor

S. Cazzaniga[1], L. Dall'Osto[1], J. Szaub[2], S. Purton[2], R. Bassi[2]
[1]Dipartimento di Biotecnologie, Università di Verona, 37134 Verona Italy, [2]Institute of Structural and Molecular Biology, University College London, London WC1E 6BT, UK

The utilization of biomass from microalgae for biofuel production is one of the key elements for the development of a sustainable energy supply. However, the economic feasibility of growing algae at industrial scale is yet to be realized. The successful implementation of biofuel production facilities requires the development of algal strains with enhanced light use efficiency. Such domestication strategy include decreasing the absorption cross section and increasing the size of metabolic sinks per chlorophyll. We applied random mutagenesis and phenotypic selection to the fast-growing strain UTEX1230 of *Chlorella sorokiniana*. Two truncated antenna mutants (TAM-2 and TAM-4) were selected and found to have half the chlorophyll content per cell and LHCII complement per PSII than the WT. In batch culture, TAM-2 showed an increased photon use efficiency, yielding a higher P_{max}at saturating light, and its cultivation in a lab-scale photobioreactor showed a higher biomass yield in dense cell suspensions than WT. These results suggest that generation of mutants with low chlorophyll content can improve light-to-biomass conversion efficiency of *C. sorokiniana* under mass culture conditions.

O7.4
Biotechnological transformation of hydrocortisone to prednisolone by coupling Streptomyces roseochromogenes and Arthrobacter simplex biocatalytic activities

O. F. Restaino, M. Marseglia, P. Diana, M. Borzacchiello, M. De Rosa, C. Schiraldi
Department of experimental medicine, Section of Biotechnology, Medical hystology and Molecular Biology

In the last years the biotechnological production of steroids by microbial transformations has became a common practice and a reliable method to produce structural tailored-cut molecules. Hydrocortisone (HC) is a pharmaceutical active molecule commercially available, its hydroxylated and dehydrogenated form, such as 16a-hydroxy-prednisolone, proved even higher anti-inflammatory activity. This latter could be obtain by whole cell biocatalyses exploiting Streptomyces roseochromogenes and Arthrobacter simplex. Initial investigations were accomplished to optimize biotransformation of HC to its 16a-hydroxy form. Results demonstrated the possibility to obtain during 15L scale fed-batch fermentations of S. roseochromogenes (30°C and pH 7), 0.804 ± 0.05 g"L-1 of 16±-OH-HC, with highly stereospecific hydroxylation. Downstream process optimization is now in progress to extract 16α-OH-HC from fermentation broths. Specifically, hydrophobic chromatography media has been compared to sole solvent extraction processes. References Donova M.V. et al. (2012). Appl Biochem Microbiol 94:1423-1447. Restaino O. F. et al. (2013) Appl Microbiol Biotechnol. 2014 Feb;98(3):1291-9.

O7.5
Phenotypic variation of the symbiotic nitrogen-fixing bacterium *Sinorhizobium meliloti* in response to the plant flavonoid luteolin investigated by Phenotype Microarray

G. Spini, F. Decorosi, G. Pallara, L. Giovannetti, C. Viti
Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA) and Laboratorio Genexpress, Università degli Studi di Firenze

The establishment of the *Sinorhizobium meliloti*-legume symbiosis depends on a complex molecular signal exchange that involves releasing flavonoid luteolin by the plant, its perception by bacterial NodD transcriptional regulator and then induction of *nod* genes., Since phenotypic changes following NodD-mediated perception of plant flavonoid still remain to be elucidate, a wide phenotypic analysis of *S. meliloti* in response to plant signal luteolin has been performed using the Phenotype Microarray (PM). PM experiments were conduced in presence and absence of luteolin, both on the wild type *S. meliloti* Rm1021 and on its mutant strain A2012 lacking the NodD regulator. The PM system allowed to test simultaneously nearly 2000 phenotypes including C, N, P, and S source utilization, growth in gradient of pH and osmolytes, and in presence of toxic chemicals., The obtained results showed a different chemical sensitivity profile for the NodD mutant strain compared to the wild-type. Moreover an interesting luteolin-induced resistance profile towards a set of compounds (biocides, intercalators, respiration-ion channels inibitors, membrane detergents, antibiotics), both NodD regulator dependent and independent, was found.

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P8.1
Effect of gender on platelet serotonin transporter expression in class III obese individuals

L. Betti[1], L. Palego[2], A. Marsili[2], F. Santini[2], A. Lucacchini[1], G. Giannaccini[1]
[1]Department of Pharmacy, University of Pisa, Pisa, Italy, [2]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

Serotonin (5-HT) is a modulator of eating behavior. However, the molecular mechanisms linking its action to body weight balance have been only partially elucidated. Since platelets are a suitable peripheral model to study 5-HT re-uptake system, we herein evaluated the expression of the platelet 5-HT transporter (SERT) by [^{3}H]paroxetine binding assay. A cohort of 32 grade III obese (OB) individuals (10 males, 22 females) without major psychiatric disorders, was recruited and classified accordingly to their body mass index (BMI). Considering the 2 groups of OB subjects, [^{3}H]paroxetine B_{max} (density, fmol/mg proteins) was reduced in platelet membranes of both vs. controls. Conversely, [^{3}H]paroxetine K_d (dissociation constant, nM) did not differ. For gender analyses, data were transformed into (B_{max})/BMI ratios to overcome both the disparity of women vs. men number and anthropometric differences between sexes. No gender-related variation concerning B_{max}/BMI ratios was observed in our subjects. The down-regulation of SERT in platelet membranes of severe human obese (BMI>40 Kg/m^2) confirms the involvement of 5-HT system in body weight gain without gender-related appreciable differences.

P8.2
Effect of feeding on plasma serotonin and tryptophan levels in untrained horses

A.M. Ferlazzo, G. Zanghì, G. Intrivici, G. Bruschetta
Dept. of Veterinary Sciences, University of Messina, Messina, Italy

Serotonin (5-HT) is a biogenic amine which play a role as an intestinal secretagogue in gastrointestinal (GI) disorders in newborn foals (1) and adult horses (2). Moreover, it is known that 5-HT containing intestinal enterochromaffin cells respond to changes in luminal contents induced by nutrients (3). On this basis, aim of this study was to investigate the effect of feeding on plasma levels of 5-HT and tryptophan in horses. N.7 mares clinically healthy, San Fratellana and crossbred, aged 7 ± 3 years, coming from a farm a.s.l., were fed with a standard diet twice a day. Compared to the fasted values, 5-HT showed significant higher levels after 3 hour postprandial ($P<0.05$). Tryptophan levels were unchanged. No differences in blood parameters were found. Further assays by sampling at longer intervals after feeding are required to confirm these preliminary data, in order to provide an useful marker of GI horse pathologies.,*1. Fink et al., (2006) Anat. Histol. Embryol., 35, 23-27, 2. Delesalle et al., (2008) Equine Vet. J., 40, 326-331, 3. Raybould et al., (2002) Gut, 51, i11-i14*

P8.3
Functional characterization of a recombinant maize allergenic chitinase

M. Volpicella[1], I. Fanizza[1], C. Leoni[1], G. Galante[1], L. Farioli[2], E. Pastorello[2], T. Naumann[3], L.R. Ceci[4]
[1]Department of Biosciences, Biotechnology and Pharmaceutics Sciences, University of Bari, Bari, Italy, [2]Unit of Allergology and Immunology, Niguarda Ca' Granda Hospital, Milano, Italy, [3]Bacterial Foodborne Pathogens and Mycology, Nat. Center Agric. Unit Res., Peoria, IL, USA, [4]Institute of Biomembranes and Bioenergetics – CNR, Bari, Italy

Food allergens are proteins belonging to a small group of about 30 families, with restricted biochemical functions. This leads to the assumption that allergens must meet specific, but not yet completely elucidated, structural features, which are at the basis of allergenicity. Their disclosure could greatly accelerate advances in the prevention, diagnosis, and therapy of allergies. In the case of food allergens, uncovering the structural basis of allergenicity would provide insights for understanding the effects of food processing and contribute to a safer management of food proteins. Among food allergens, those of plant origin constitute an essential part. Plant chitinases are a good example of food allergenic proteins for which structural analysis of allergenicity has only been carried out partially. Here we report characterization of the activity of a recombinant maize chitinase. The mature protein has been assayed for allergenicity by immunoblot assay. On plate colorimetric assays and microcalorimetry measurements are in progress at increasing temperatures and low pHs to evaluate the activity retention of the molecule at extreme conditions.

P8.4
Inhibitory effects of different catechin formulations on tumor cells

M. Giannaccini[1,2], G. Cirillo[3], O. Vittorio[4], L. Dente[2], V. Raffa[1,2]
[1]Inst. Life Science,Scuola Superiore Sant'Anna, Pisa, Italy, [2]Dept. of Biology, University of Pisa, via Abetone e Brennero 4, Pisa, Italy, [3]Dept of Pharmacy, Health and Nutrition Sciences, Univ. of Calabria, Arcavacata di Rende (CS), Italy, [4]Australian Centre for Nanomedicine, Univ. of New South Wales, New South Wales, Australia

Catechins are natural flavonoids found in a variety of plant-derived beverages and edible vegetables. High concentrations of catechin can be found in green tea, red wine, chocolate and fruits. Consumption of catechins has been associated with beneficial effects including antioxidant activity, blood vessel expansion, decreased LDL oxidation, and reduced susceptibility to various cancer types. Natural catechin is very unstable and easily oxidable, but pharmacokinetics profiles of this drug could be easily modified by exploiting recent advances in the nanomedicine field. Specifically, two different nanoformulations were tested: gelatine-catechin conjugates and gelatine-catechin conjugates incorporating carbon nanotubes (CNT). Melanoma is a very aggressive cancer and a leading cause of death due to its highly metastatic ability. We investigated inhibitory effects of different catechin formulations on the invasive potential of human melanoma A375 cells by performing cytotoxicity, cell proliferation and cell invasion assays. In parallel, to investigate in vivo anti-tumor and anti-metastasis effects, we are employing transgenic zebrafish as model for human melanoma cell xenotransplants.

P8.5
In vitro modulation of pancreatic insulin secretion, extrapancreatic insulin action, and peptide glycation by Curcuma longa aqueous extracts

V. Kasabri[1], P.R. Flatt[2], Y.H. A. Abdel-Wahab[2]
[1]Dept Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, The University of Jordan, Amman, Jordan, [2]School of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland BT52 1SA, UK

Objective: Medicinal, edible and aromatic plants have been used as folk remedies in traditional treatments worldwide. This study investigates the antidiabetic efficacy and action mode of Curcuma longa Linn. (Zingiberaceae). Methods: Effects of aqueous extracts (AEs) of C. longa on insulin secretion and action were studied using BRIN BD11 cells and 3T3L1 cells, respectively. In vitro models were employed to evaluate effects on starch digestion using α-amylase/amyloglucosidase and protein glycation. Results: C. longa AEs stimulated basal insulin output and potentiated glucose-stimulated insulin secretion concentration-dependently in the clonal pancreatic beta cell line, BRIN-BD11 ($p<0.001$). The insulin secretory activity of plant extract was abolished in the absence of extracellular Ca2+ and by inhibitors of cellular Ca2+ uptake, diazoxide and verapamil, ($p<0.001$). Furthermore, the extract increased insulin secretion in depolarised cells and further augmented insulin secretion triggered by IBMX, tolbutamide and glibenclamide. C. longa AEs lacked insulin mimetic activity but enhanced insulin-stimulated glucose transport in 3T3L1 adipocytes by 370% ($p<0.001$).

Similar to aminoguanidine, C. longa AEs (1-50mg/ml) effected concentration-dependent inhibition of protein glycation (24-70% inhibition, $p<0.001$) in vitro. In bioassays of enzymatic starch digestion, C. longa AEs lacked inhibitory effects on α-amylase and α-glucosidase, unlike acarbose, the classical reference drug. Conclusion: This study has revealed that water soluble bioactive principles in C. longa AEs stimulate basal- and potentiate glucose evoked- insulin secretion, enhance insulin action and inhibit insulin glycation but not starch digestion. C. longa AEs may provide new opportunities for the combinatorial treatment/ prevention of diabetes.

P8.6

Bioactive peptides with cardiovascular protective activity from wheat grains

E. Leoncini[1], G. Dinelli[3], C. Prata[2], L. Massaccesi[1], M. Malaguti[1], V. Bregola[3], S. Bosi[3], J. Fiori[2], R. Gotti[2], S. Hrelia[1]

[1]Department for Life Quality Studies, [2]Department of Pharmacy and Biotechnology, [3]Department of Agricultural Sciences – Alma Mater Studiorum-University of Bologna, Italy

Food bioactive peptides are currently investigated as they display functional activities for health promotion. Although high in carbohydrates, cereals contain substantial amount of proteins thus representing a possible dietary source of bioactive peptides. In the search of bioactive peptides in different wheat varieties a 7 kDa peptide was observed by means of HPLC-UV and nanoLC-nanoESI-QTof analyses in wheat flour extracts. Through data base searches the 7 kDa signal was ascribed to a Non-specific lipid-transfer protein (NsLTP) type 2 (sequence coverage of 92%). As no data regarding NsLTP type 2 biological activity in mammalian cells are present in the literature, experiments were aimed at the analysis of potential mechanisms by which this peptide can contribute to cardiovascular health using HUVEC cell cultures. In particular antioxidant activity and cytoprotective effects following oxidative or inflammatory stimulation were evaluated. Moreover, NsLTP type2 was also analysed for its ability to inhibit Angiotensin converting enzyme, a therapeutic target for the treatment of hypertension.,This work was supported by BACCHUS project (FP7 European Commission Grant Agreement 312090).

P8.7

Cardio and vascular protective activities of Olea Europea and Hibiscus extratcs

M. Malaguti[1], M. Micucci[2], T. Gallina Toschi[3], P. Comandini[3], R. Budriesi[2], S. Hrelia[1]

[1]Dipartimento di Scienze per la Qualità della Vita, Università di Bologna, [2]Dipartimento di Farmacia e Biotecnologie, Università di Bologna, [3]Dipartimento di Scienze e Tecnologie Agro-Alimentari, Università di Bologna

Olive tree leaves are a source of bioactive compounds, as oleuropein, flavonoids, and triterpenes. Hibiscus, used for centuries in the treatment of hypertension and hyperlipidemia, is known to be a rich source of anthocyanins. We evaluated the cardiovascular protective effects of *Olea Europea* leaves (OE) and *Hibiscus* flower (HE) extracts and a 26:4 w/w combination of the two (MIX). The extracts were characterized in their bioactive composition by HPLC-DAD-MS analysis. OE, HE and MIX reduced intracellular reactive oxygen species formation and improved cell viability following oxidative stress in dose dependent manner in HUVEC cell cultures. In isolated guinea pig left and right atria and aorta the effect on cardiovascular parameters was evaluated. OE, HE and MIX showed a similar negative inotropic effect. MIX, in contrast to OE and HE, showed a significant negative chronotropic activity. On vascular smooth muscle, OE and HE reduced the contraction induced by 80 mM K^+ in a dose-dependent manner and MIX evidenced a miorelaxant activity higher than OE and HE. So, the combination of OE and HE could represent a nutraceutical approach in the protection of cardio and vascular health.

P8.8

Nutraceutical properties of Tuscan chestnut flours: beneficial effects on skeletal muscle atrophy.

E. Meacci[1], A. Frati[1], C. Marinelli[2],M. Migliorini[2], M. Garcia-Gil[3] D. Landi[1], L. Fontana[1], F. Pierucci[1]

[1]Dept of Experimental Clinical and Biomedical Sciences, University of Florence, ITALY, [2]Metropoli, Special Agency of the Chamber of Commerce of Florence, ITALY, [3]Department of Biology, University of Pisa, ITALY

Plants contain a wide range of non-nutritive phytochemicals, many of which have protective or preventive properties for human diseases. The aim of the present work has been to investigate the nutraceutical properties of sweet chestnut flour extracts obtained from fruits collected from 7 geographic areas of Tuscany (Italy), and their ability in modulating skeletal muscle atrophy. We found that the cultivars from different geographic areas are characterized by composition and quantity of various nutrients and specific bioactive components, such as tocopherols, polyphenols and sphingolipids. The nutraceutical properties of chestnut sweet flours have been evaluated in myotubes induced to atrophy by serum deprivation or dexamethasone. We found that the pretreatment with both total extract of tocopherols and sphingolipids is able to counterbalance cell atrophy attenuating protein degradation and the increase in expression of a muscle-specific atrophy marker. By contrast, polyphenol extracts were not able to prevent atrophy. This is the first evidence that chestnut sweet flour is a natural source of specific bioactive components with a relevant role in the prevention of cell degeneration and maintaining of skeletal muscle mass, opening important implications in designing appropriate nutritional therapeutic approaches to skeletal muscle atrophy.

P8.9

In vitro assessment of Novel nutraceutical formulation: hepatocyte based model exploiting time lapse video-microscopy

A. Stellavato, A.V.A. Pirozzi, F. de Novellis, I. Scognamiglio, M. De Rosa, C. Schiraldi

Dept Experimental Medicine, Section Biotechnology Medical Histology and Molecular Biology Second University of Naples, Italy

Non Alcoholic Fatty Liver Disease (NAFLD) is a pathological situation caused by an increase of intracellular fatty acids followed by inflammation and cell necrosis [1]. Today there isn't a specific therapy only nutritional guides lines are given to prevent to disease progression. The aim of this research, was to assess the potential of natural active ingredients and their formulations on a NAFLD in vitro model. Specifically, the effect of three potential nutraceuticals was evaluated, namely Vitamin E , Silimarin and Curcumin [2]. Human hepatic cancer cells (HepG2 cell line) where treated with a mix of oleic and linoleic acids, towards induction of NAFLD in vitro [3], then the amount of intracellular fatty acids produced was evaluated by Oil Red staining. The ability of the selected potential nutraceuticals and eventually their combination to reduce fatty acid deposit was evaluated. The peroxisome proliferator-activated receptors (PPARαand γ), were evaluated as biomarkers. Studies on oxidative stress and lipid peroxidation are currently in progress. *References 1. Jain M et al.J Pharm Pharmacol. 2012 Jun;64(6):888-96. 2. Kang O.H et al. EurRev Med Pharm Sci 2013;17: 2578-2586.*

P8.10

Characterization and evaluation of potential anti-angiogenic activity of tomato miniproteins and lycopene

D. Treggiari[1], G. Zoccatelli[1], B. Molesini[1], A. Dalbeni[2], M. Degan[2], P. Minuz[2], T. Pandolfini[1]

[1]Dept. Biotechnology, Univ. Verona, Italy, [2]Dept. Medicine, Univ. Verona, Italy

Angiogenesis, the formation of new blood vessels, is critical to tumour growth and inflammation. The discovery of angiogenesis inhibitors disclosed effective therapeutic strategies. Dietary food compounds

have received increasingly attention in disease prevention. The fruit of tomato is rich in compounds with potential anti-angiogenic activity such as lycopene and cystine-knot miniproteins (TCMPs). Aim of this study was to characterize*in vitro* the mechanism of the anti-angiogenic activity of TCMPs and lycopene. Using human umbilical vein endothelial cells (HUVEC), we found that TCMPs inhibit by approximately 50% the increase in cell migration induced by VEGF-A and significantly decrease the nitric oxide (NO) formation. On the contrary, lycopene increased NO production in VEGF-A- treated HUVEC. Fruits from several tomato varieties were analyzed for their TCMPs content, observing variations in TCMPs levels particularly in fresh-market varieties. Moreover, we identified putative cystine-knot proteins in other *Solanaceae* species (i.e. .tobacco and eggplant). These findings suggest that TCMPs may be of pharmacological interest.

O8.1
Neuroprotective effects of sulforaphane on methylglyoxal-induced glycation in SH-SY5Y cell line

C. Angeloni, M.C. Barbalace, B. Rizzo, D. Fabbri, M. Malaguti, S. Hrelia
Department for Life Quality Studies - Alma Mater Studiorum, University of Bologna (Italy)

Glycation, an endogenous process that leads to the production of advanced glycation end products (AGEs), plays a role in the etiopathogenesis of Alzheimer's disease (AD). Methylglyoxal (MG) is the most potent precursor of AGEs. Recently, it has been demonstrated that AGEs cause a reduction in brain derived neurotrophic factor protein (BDNF), whose dysregulation is related to AD development. Sulforaphane (SF), an isothiocyanate found in Cruciferous vegetables, is known for its chemopreventive, cardioprotective and neuroprotective actions. Aim of this study was to investigate the role of SF in counteracting MG induced damage in SH-SY5Y neuroblastoma cells. SF counteracted MG-induced neuronal death and apoptosis as measured by MTT assay, LDH release and caspase 3 activity. SF significantly reduced intracellular ROS and increased GSH levels in MG treated cells. SF inhibited the phosphorylation of the pro-apoptotic MAPK kinases induced by MG, increased the expression and the activity of the MG-detoxifying enzymes glyoxalase 1 and glyoxalase 2 and increased BDNF expression, demonstrating a pleiotropic role in counteracting AD. This work was supported by MIUR-FIRB (project RBAP11HSZS)

O8.2
Nutritional values and radical scavenging capacities of lentil (*Lens culinaris* Medik) seeds in Valle Agricola district, Italy

N. Landi, M. C Mezzacapo, A. Di Maro
Dept of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Caserta, Italy

The local cultivars are tied up to a tradition of transformation for "homemade" products. Therefore it is interesting to consider possible intervention strategies to preserve genetic variability of local cultivars, according to the traditional approach of biochemical and nutritional valorisation. Lentil (*Lens culinaris* Medik) cultivation has a positive impact on agriculture and the environment. In this framework we have investigated nutritional values and metabolic profile of Lens culinaris Medik seeds grown in Valle Agricola because there are no nutritional data available on them. The analysis is focused on the content of moisture, ash, total protein, carbohydrates, lipids, amino acid composition, total polyphenols and antioxidant activity by ORAC assay. In particular the content of total proteins, lipids and carbohydrates in the lentils is 26.27±0.52 g/100g, 2.5±0.2g100g, 56.72g/100g of flour respectively, while the ashes 3.50±0.00g/100g, moisture 8.60±0.20 g/100g of flour. The amino acid analysis shows a high content of essential amino acids as leucine, isoleucine, lysine threonine, whereas methionine and cysteine are limiting amino acids. The analysis, finally, have shown that the seeds of Valle Agricola lentil have substantial antioxidant power thanks to the presence of polyphenols (23.14±0.76 mg/g of flour), equal to 6.540,96±203.69 µmol TE/100 g (from ORAC assay).

O8.3
Protective effect of apple polyphenols on methylglyoxal-induced glycation of human High density lipoproteins

G. Ferretti[1], I. Turco[1], T. Bacchetti[2]
[1]Department of Clinical Science and Odontostomatology, UNIVPM, Ancona, Italy, [2] Department of Life Science, UNIVPM, Ancona, Italy

Introduction: Methylglyoxal (MG) is involved in the molecular mechanisms of diseases associated with oxidative stress and promotes advanced glycation end products (AGEs) formation. We have previously demonstrated that HDL are susceptible to MG-induced glycation (1). Aim of the study was to investigate the protective effect of apple polyphenol-rich extracts against MG-induced glycation of human HDL. **Methods:** HDL isolated from human plasma were incubated with MG (0.2-1.0mmol/L) in the presence or absence of fruit extracts. MG-induced glycation of HDL treated in the different experimental conditions was quantified as the increase of fluorescent AGEs (370ex/440em). **Results:** Apple polyphenol-rich extracts inhibited MG-mediated formation of fluorescent AGEs in a dose-dependent manner both in HDL. Moreover, the presence polyphenol-rich extracts reduced apoprotein alterations induced by glycation. **Conclusions:** Polyphenol-rich extracts protect HDL against alteration induced by MG. Our data support the hypothesis that apple polyphenols could be used as supplements for prevention of diabetes and its complications. 1) Bacchetti T. et al. G*lycation of human high density lipoprotein by methylglyoxal: effect on HDL-paraoxonase activity*. Metabolism. 2014 Mar;63(3):307-11.

O8.4
Physiological consequences of beta-lactoglobulin adsorption on hydrophobic surfaces: making a bad food allergen even worse?

M. Miriani[1], R. Gianoglio Bernardi[1,2], H. Frøkiær[2], F. Bonomi[1], S. Iametti[1]
[1]Section of Chemical and Biomolecular Sciences, DeFENS, University of Milan, Italy, [2]Department of Veterinary Disease Biology, Section of Experimental Animal Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Beta-lactoglobulin (BLG) is abundant in bovine whey and is a major food allergen. The physiological consequences of technological treatments of BLG are much debated, also because multiple mechanisms seem to be involved in BLG recognition and uptake. Since food protein allergens are consumed in the presence of other food components, we aimed at understanding the consequences of conformational changes ensuing from the interaction of BLG with hydrophobic interfaces (such as oil-in-water emulsions), and how these can affect its immunoreactivity and uptake by cells of the immune human system. BLG increases its immunoreactivity after interaction with the interface, and the immunoreactivity of interface-bound BLG remains high after trypsin hydrolysis, at least when compared with the protein hydrolyzed in solution. However, the physical state of BLG does not influence the rate of its uptake by antigen presenting cells, e.g. monocytes, although different uptake pathways seem to operate for free and interface-bound BLG. These results highlight the importance of the protein physical state with respect to physiologically relevant properties, with significant practical implications.

O8.5
Docosahexaenoic acid influences cholesterol metabolism in cancer cells

P. A. Corsetto[1], C. Riganti[2], G. Montorfano[1], P. Roderi[1], A. M. Rizzo[1]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Italy, [2]Dipartimento di Oncologia, Università degli Studi di Torino, Italy

Omega-3 fatty acids (ω3-FA), such as docosahaesanoic acid (DHA),

attenuate growth and induce apoptosis in a variety of cancer cell lines derived from colonic, pancreatic, prostate, and breast cancers. In addition, recent findings indicate that ω3-FA act synergistically with chemotherapeutic agents and may be used to enhance radiosensitivity. The mechanisms underlying the anti-tumour effects of ω3-FA are complex. Lipids are likely to be utilized for membrane composition, protein modification and signal transduction as a second messenger, all of which are crucial in cancer cells. We propose that incorporation of ω3-FA alters the profile of lipid composition influencing EGFR downstream signal transduction in breast cancer cells. We demonstrated that DHA is incorporated and metabolized in breast cancer plasma membrane, especially in lipid rafts, with different specificity for the phospholipids moiety, altering their structure. Of note is the observation that the treatment with DHA modifies cholesterol synthesis, reducing farnesyl or geranyl moiety. Many acylated proteins directly interact with plasma membrane, especially lipid rafts, by their saturated moieties. Membrane incorporation of DHA induces alterations in the lateral localization of EGFR and a decrease of EGFR downstream signal transduction. In particular, DHA induces down-regulation in the EGFR-Ras-ERK1/2 and PI3K pathways.

9 - Epigenetics and Epigenetic Therapies

P9.1
Social defeat stress alters the expression of psychiatric disorders related genes in mouse brain cortex

S. Mansueto[2], D. Punzo[1,3], W. Durso[2], C. Caterino[2], E. Florio[1], L. Coretti[2], S. Keller[1,2], A. Usiello[3], L. Chiariotti[1,2]
[1]Dipartimento di medicina molecolare e biotecnologie mediche, Università Federico II, Naples, Italy, [2]Istituto di endocrinologia e oncologia sperimentale IEOS, CNR, Naples, Italy, [3]Ceinge biotecnologie avanzate, Naples, Italy

Exposure to stressful or traumatic life events is one of the strongest risk factors for a number of psychiatric disorders. One of the proposed mechanism is alterations in gene transcription, and thus in neural function, caused by epigenetic modifications, such as DNA methylation and hydroxymethylation. To better understand this relationship, we want to verify whether the expression of genes involved in DNA hydroxymethylation pathway and stress response changes in mice submitted to social stress. To this aim, we extracted RNA from brain cortex of two months-old mice submitted to social defeat stress (based on the introduction of an unfamiliar resident into the home cage) for two cycles of 10 days. We analyzed the expression of genes involved in 5-hydroxymethylcytosine generation (Tet1, Tet2, Tet3), in psychiatric disorders (MeCP2, BDNF, Cdkl5) and in glutamate methabolism (Slc1a3 and Grm5) by real-time RT-PCR. We found that the expression of Tet genes does not change in stressed mice compared to control, whereas BDNF and Grm5 are up-regulated and MeCP2, Cdkl5 and Slc1a3 are down-regulated in stressed mice. Epigenetic state of promoters of these genes is under investigation.

P9.2
Nhp6 as regulator of gene transcription

F. Di Felice[1], D. Durano[1], A. Lukacs[1], G. Micheli[2], G. Camilloni[1]
[1]Department of Biology and Biotechnology "C. Dawin", Sapienza University of Rome, [2]IBMN, CNR Rome

Increase or decrease of the histone content, reflects profound changes in gene expression, genome stability and aging and is crucial for cell life. Histone amount is tightly determined through a series of different levels of regulation. Any alteration of these controls, leads to histone number variation and altered nucleosome occupancy that recently has been proposed as a genome wide level of epigenetic regulation. Significant histone reduction and transcription alterations have been shown for cells lacking HMGB1 proteins, both in mammals and in *S.cerevisiae*. Yeast Nhp6p belong to the HMGB1 family. These proteins are involved in transcriptional regulation and initiation or elongation processes. Mutant strain *nhp6ab* shows a slow growth phenotype, increased genome instability and shortened life span. We focused our attention on the histone decrease. The aim of this work is to investigate the molecular mechanisms responsible for the histone decrease found in *nhp6ab* mutants, with particular attention on transcriptional regulation. We will provide evidences for Nhp6p as regulator of histone genes transcription, stabilizing the nucleosome around the transcriptional start site.

P9.3
Spreading Depolarization in nervous cells:DNA methylation and Preconditioning phenomenon

D. Drongitis, A. Donizetti, S. Rainone, M. Piscopo, L. Fucci
Dept of Biology, Federico II Univ., Naples, Italy

Mammalian brain can acquire tolerance to an injurious insult by non-injurious stimulus (like *Spreading Depolarization*(SD)) inducing the so-called "Preconditioning phenotype". Previously we provided evidence of correlation between *Spreading Depolarization*-induced*Preconditioning*and epigenetic modifications in rat brains; in particular our results demonstrated changes in lysine methylation of H3 histone at neuroprotective genes and retrotransposon sequences. We demonstrate that SD induces DNA hypermethylation of retrotransposon sequences corroborating the hypothesis of global genomic silencing. These data are in accordance with the increased expression level of all DNA methyltrasferase genes observed in preconditioned brain hemispheres. We also developed an *in vitro* human neuronal cell model system by using SH-SY5Y cell line. This approach allows us to investigate the *Preconditioning* effects on a homogeneous human neuronal cell population. We demonstrate that both undifferentiated and differentiated SH-SY5Y cells show resistance to H_2O_2-induced cell death when preconditioned by KCl-induced depolarization.

P9.4
Unexpected genome-wide DNA methylation stability across plant populations adapted to divergent habitats

E. Trucchi[1], R. Flatscher[1,2], M.T. Lorenzo[1], B. Frajman[2], P. Schönswetter[2], O. Paun[1]
[1]Department of Botany and Biodiversity Research, University of Vienna, Austria, [2]Institute of Botany, University of Innsbruck, Austria

Across heterogeneous environments, variation in biotic and abiotic conditions often leads to the formation of distinct populations adapted to their specific habitat. In the *Heliosperma pusillum* s.l., the lower elevation *H. veselskyi* has a dense sticky indumentum and inhabits rock overhangs and shallow caves whereas its closest relative, the alpine *H. pusillum,* is glabrous and occurs on creeks and moist calcareous screes. Both standard and bisulfite-converted RAD sequencing have been employed to search for the hard-coded genetic and/or soft-coded epigenetic determinants of the phenotypic and ecological differentiation. Over 1200 high quality SNPs data show that the two species are genetically intermixed, suggesting recent and recurrent origins. However, no loci have been identified to be (or linked to loci) under divergent selection. Preliminary data on DNA methylation pattern show a general lack of differentiation between the two species (only 2 outliers loci found) supporting instead a much more stable methylation scenario than expected. If confirmed, a "hard-coded" function for the methylome in shaping evolutionary processes should also be taken into account.

O9.1
Genetic and environmental factors linked to DNA methylation in colorectal cancer revealed by artificial neural networks

F. Coppedè[1,2], E. Grossi[3,4], A. Lopomo[1,5], R. Spisni[6], M. Buscema[4,7], L. Migliore[1,2]
[1]Department of Translational Research and New Technologies in Medicine and Surgery, Division of Medical Genetics, University of Pisa, Pisa, Italy, [2]Istituto Toscano Tumori (ITT), Florence, Italy, [3]Bracco Foundation, Milan, Italy, [4]Semeion Research Center, Rome, Italy, [5]Doctoral School in Genetics, Oncology, and Clinical Medicine, University of Siena, Siena, Italy, [6]Department of Surgery, Medical, Molecular, and Critical Area Pathology, University of Pisa, Pisa, Italy, [7]Department of Mathematical and Statistical Sciences, University of Colorado at Denver, CO, USA

In the present study we applied Artificial Neural Networks (ANNs) to identify genetic and dietary/lifestyle factors linked to *MLH1*, *APC*, *CDKN2A*^{INK4A}, *MGMT*and *RASSF1A* gene promoter methylation in sporadic colorectal cancer (CRC). We applied the Auto Contractive Map-Auto-CM algorithm (Auto-CM), a special kind of ANNs able to define the strength of the association of each variable with all the others and to visually show the map of the main connections of the variables and the basic semantic of their ensemble. Our analysis, performed on 83 sporadic CRC samples, revealed a strong connection between the low methylation levels of the five CRC genes and the *MTR*2756AA genotype. High *MLH1*methylation levels were linked to high *CDKN2A*^{INK4A} methylation levels, low *APC*methylation levels, and right colon location, while low *MLH1*methylation levels were linked to male gender. Advanced age and

adenoma stage were linked to *MGMT* hypermethylation, while the major contributor to the *RASSF1A* methylation status was the *MTR*genotype. Several other connections were revealed, including those between dietary and lifestyle factors and the methylation levels of the five studied CRC genes.

O9.2

In vivo selection of *JARID* histone demethylases inhibitors and their use to enlighten the biological role of these enzymes in yeast and mammalian cells with focus on transcriptional regulation

S. Danovska[1], S. Pippa[1], V. Licursi[1], C. Mannironi[2], V. Vapore[1], M. Proietto[1], F. Bufalieri[1], E. Cundari[2], A. Alagia[1], T. Rinaldi[1], V. Famiglini[3], A. Coluccia[3], G. La Regina[3], R. Silvestri[3], R. Negri[1]

[1]Istituto Pasteur Fondazione Cenci Bolognetti, Dipartimento di Biologia e Biotecnologie C. Darwin, Sapienza Università di Roma, [2]Istituto di Patologia e Medicina Molecolare CNR, Roma, [3]Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma

We recently discovered in *S. cerevisiae* an interesting conditional negative genetic interaction between the unique *JARID* histone demethylase Jhd2, responsible for H3K4 demethylation, and Not4, a protein involved in several different regulatory processes, including transcriptional regulation, RNA stability and Jhd2 degradation. The double deletion mutant Δjhd2/Δnot4 is hypersensitive to rapamycin and its sensitivity is promptly suppressed by episomal Jhd2 expression in the double deletion strain. This genetic interaction is an ideal system for *in vivo* screening of inhibitors specific for JARID demethylases. In a pilot screening on 45 candidate small molecules, we identified a compound which specifically inhibits Jhd2 *in vivo*, leading to a consistent increase in trimethyl-H3K4. The compound inhibits human JARID 1B and 1D *in vitro* and shows a strong cytostatic effect, a mild cytotoxicity and a selective increase of trimethyl-H3K4 in HeLa cells. We better characterized the inhibitor's effects in yeast and mammalian cells, with focus on transcriptional effects. The results in yeast enlighten a role of Jhd2 in regulating a set of genes transcriptionally induced upon diauxic shift.

O9.3

Genetic and epigenetic implications of the *MTHFR* gene in cardiovascular disease

M. Denaro[1], C. Modena[1], F. Coppedè[1], F. Pirrami Collarino[2], M. Ferrari[2], L. Migliore[1]

[1]Department of Translational Research and New Technologies in Medicine and Surgery, Medical Genetics Laboratory, University of Pisa, Italy, [2]Department of Translational Research and New Technologies in Medicine and Surgery, Unit of Vascular Surgery, University of Pisa, Italy

Cardiovascular diseases (CVD) represent the most common cause of death in developed and developing countries. The clinical manifestations are the result of atherosclerosis, a chronic degenerative disease of the arteries. Several studies have focused on the relationship between epigenetics and CVD risk factors, suggesting that homocysteine (hcy) is the most implicated biomarker associated with an alterated DNA methylation pattern. One of the major contributors to the elevation in plasma hcy levels is the methylenetetrahydrofolate reductase (*MTHFR*) 677TT genotype. Our findings highlight a significant difference in hcy levels in patients that underwent to carotid endarterectomy with respect the controls, we also observed significant decreased methylation levels in the *MTHFR* gene promoter in the patient group. In addition, a significant difference between *MTHFR* methylation levels in peripheral blood lymphocytes (average 31,1%) with respect to atherosclerotic plaques (average 18,1%) was seen. We are currently investigating possible genetic and environmental factors linked to *MTHFR* methylation in CVD patients.

O9.4

Analysis of the interaction between LSD1, lysine-specific demethylase 1, and protein kinase CK2

S. Sarno, G. Cozza, C. Girardi, L.A. Pinna

Department of Biomedical Sciences and CNR Institute of Neurosciences, Viale G. Colombo, University of Padua, Padua, Italy

LSD1 is a FAD-dependent amine oxidase implicated in epigenetic regulation, catalyzing the specific removal of the methyl groups from mono-(me1) and dimethylated-(me2) K4 and K9 of histone H3. We identified LSD1 as a new substrate of protein kinase CK2, a highly pleiotropic enzyme composed of two catalytic α/α' and two regulatory β subunits and we identified three CK2 phosphosites in the N-terminal region of LSD1. Moreover we demonstrate that LSD1 and CK2 interaction occurs *in vivo*. High expression of CK2 and LSD1 has been reported in several tumor types therefore their interaction can play a role in cellular transformation. A protein-protein docking analysis between LSD1 and αCK2 suggests a productive interaction involving a large surface (980 Å) indicating that LSD1-CK2 complex is quite stable. To obtain quantitative data for the binding properties of LSD1 with CK2, the interaction between the two proteins has been analyzed by surface plasmon resonance using a BIACORE T100 instrument. Kinetic parameters revealed that LSD1 interacts very strongly with αCK2, and data obtained with αCK2 mutants confirms protein-protein docking analysis. Supported by AIRC (grant IG-10312)

O9.5

Gene specific DNA methylation analysis in peripheral blood as potential biomarker for Alzheimer's Disease

A. Stoccoro[1], P. Tannorella[1,2], P. Bosco[3], G. Tognoni[4], U. Bonucelli[4,5], L. Migliore[1], F. Coppedè[1]

[1]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy, [2]Department of Biology, University of Pisa, Pisa, Italy, [3]IRCCS, Oasi Maria SS Institute for Research on Mental Retardation and Brain Aging, Troina, Italy, [4]Department of Neuroscience, Pisa University Hospital, Pisa, Italy, [5]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

DNA methylation is an epigenetic mark altered in AD brains and there is increasing interest in searching for peripheral epigenetic biomarkers of AD. We searched for peripheral blood DNA methylation modifications of key genes involved in DNA methylation and amyloid beta production in almost 100 AD patients, 50 Mild Cognitive Impairment (MCI) individuals, a preclinical condition of AD, and 100 matched controls. We also correlated the obtained methylation levels with biomarkers of folate metabolism that is involved in DNA methylation reactions. Methylation levels of *BACE1* promoter and exon 1, *DNMT3B*, *DNMT1* and *PSEN1*gene promoters are the same in AD, MCI and control individuals. Significant differences in the methylation levels of the *MTHFR* gene were observed among the three groups. Furthermore, we observed a trend of decreasing methylation status of the *MTHFR* gene with increasing plasma homocysteine levels. Present data support a role for *MTHFR* as an AD epigenetic biomarker. The study was supported by the Italian Ministry of Health (GR-2009-1606229; FC Principal Investigator).

10 - Human Genetic and Genomic Diversity

P10.1

When data sharing gets close to 100%: what ancient human DNA studies can teach to the open science movement

P. Anagnostou[1,2], M. Capocasa[2,3], N. Milia[4], G. Destro Bisol[1,2]

[1]Università di Roma "La Sapienza", Dipartimento di Biologia Ambientale, Rome, Italy, [2]Istituto Italiano di Antropologia, Rome, Italy, [3]Università di Roma "La Sapienza", Dipartimento Biologia e Biotecnologie "Charles Darwin", Rome, Italy, [4]Università di Cagliari, Dipartimento di Biologia Sperimentale, Cagliari, Italy

This study analyzes sharing rates and ways of data regarding mitochondrial, Y chromosomal and autosomal polymorphisms in a total of 162 papers on human ancient DNA published between 1988 and 2013. The estimated sharing rate is not far from completion (97.5% ± 2.1%) and higher than observed in other fields of genetic research. A *questionnaire*-based survey suggests that the authors' awareness of the importance of openness and transparency for scientific progress is a fundamental factor for the achievement of the high sharing rate. Our study conveys three important messages. First, we provide evidence that researchers' motivations to share are as much necessary as stakeholders' policies and norms. Second, to make data sharing robust and effective it is important to pay attention not only to the quantity of data available but also to the way of data sharing, encouraging practices which maximize data accessibility and preservation. Finally, the case of human ancient DNA studies demonstrates that data sharing and, more in general, openness to scientific inquiry can help build rigorous and reliable scientific practices even in the presence of complex experimental challenges.

P10.2

Expression status of candidate genes in mesothelioma tissues and cell lines

E. Barone[1], O. Melaiu[1], E. Melissari[2], L. Mutti[3], E. Bracci[1], C. De Santi[1], C. Iofrida[2], M. Di Russo[2], A. Cristaudo[4], A. Bonotti[4], M. Cipollini[1], E. Paolicchi[1], S. Garritano[5], R. Foddis[4], M. Lucchi[6], S. Pellegrini[2], G. Figlioli[1], L. Pellè[1], F. Gemignani[1], S. Landi[1]

[1]Department of Biology, University of Pisa, Pisa, Italy, [2] Department of Surgical, Medical and Molecular Pathology and of Critical Care, University of Pisa, Pisa, Italy, [3] Laboratory of Clinical Oncology, Vercelli National Health Trust, Vercelli, Italy, [4] Section of Occupational Medicine, School of Medicine and Surgery, University of Pisa, Pisa, Italy, [5]Laboratory of Computational Oncology, Centre for Integrative Biology, CIBIO, University of Trento, Trento, Italy, [6] Cardiac and Thoracic Department, Division of Thoracic Surgery, University of Pisa, Italy

Malignant pleural mesothelioma (MPM) is a cancer of the pleural cavity with a poor prognosis. In order to broaden the knowledge on its mechanisms of development, a literature review of transcriptomic studies was recently performed. Only 119 genes were deregulated in at least two different publications, highlighting a poor consistency amongst different studies. In the present work, the expression status of these genes was further investigated. The expression level of 119 putatively deregulated genes was measured and compared between 15 MPM and 20 non-malignant mesothelial tissues through quantitative Real Time PCR. Positive genes were further evaluated in two epithelioid MPM cell lines and compared to a non-MPM human cell line. Twenty-nine genes were found deregulated in tissues and in at least one MPM cell line. Patients whose MPM tissues expressed mRNA levels of *BIRC5, DSP,*and*NME2* over the median showed a statistically significant shorter overall survival. Although MPM is a poorly studied cancer, some features are starting to emerge. Novel cancer genes for MPM could be further investigated, in particular those involved in cell-cell and cell-matrix interaction pathways.

P10.3

Traces of Medieval migrations in a socially-stratified population from Northern Italy. Evidence from uniparental markers and deep-rooted pedigrees

A. Boattini[1], S. Sarno[1], P. Pedrini[1], C. Medoro[1], M. Carta[1], S. Tucci[1,2], G. Ferri[3], M. Alù[3], D. Luiselli[1], D. Pettener[1]

[1]Laboratorio di Antropologia Molecolare, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Bologna, Italy, [2]Dipartimento di Biologia ed Evoluzione, Università di Ferrara, Ferrara, Italy, [3]Dipartimento di Medicina Diagnostica, Clinica e di Sanità Pubblica, Università degli Studi di Modena e Reggio Emilia, Modena, Italy

Social factors had a critical role in determining the genetic structure of Europe. Therefore, socially-stratified populations may help to focus on specific episodes of European demographic history. In this study we use uniparental markers to analyse the genetic structure of *Partecipanza* in S. Giovanni in Persiceto (Northern Italy), a peculiar institution whose origins date back to the Middle Ages and whose members form the patrilineal descent of a group of founder families. From a maternal point of view (mtDNA), *Partecipanza* is genetically homogeneous with the rest of the population. However, we observed a significant differentiation for Y-chromosomes. In addition, by comparing 17 Y-STR profiles with paternal pedigrees, we estimated a Y-STR mutation rate of $3.90 * 10^{-3}$ and an average generation duration time of 33.38 years. When we used these values for tentative dating, we estimated 1,300-600 years ago for the origins of *Partecipanza*. These results, together with a peculiar Y-chromosomal composition and historical evidence, suggest that Germanic populations settled in the area during the Migration Period and may have had an important role in the foundation of this community.

P10.4

Identification and genomic characterization of HERV-K (HML-10) in human genome GRCh37/hg19 assembly

M. Cadeddu[1], N. Grandi[1], L. Vargiu[1,2], P. Rodriguez-Tomé[2], J. Blomberg[3], E. Tramontano[1]

[1]Dept Life and Environmental Sciences, University of Cagliari, Cagliari, Italy, [2]Center for Advanced Studies, Research and Development in Sardinia, CRS4, Pula (Cagliari), Italy, [3]Section of Virology, Dept Medical Sciences, Uppsala University, Sweden

Human genome contains around 3300 Endogenous Retroviruses including the HML1-10 clade. Currently, the sole identified HML-10 sequence, HERV-K(C4) inserted within the human complement C4 gene in an antisense orientation, has been hypothesized to be involved in modulation of C4 expression, defense against retroviral infections and protection against autoimmune diseases. Using the RetroTector software on the human genome GRCh37/hg19 assembly we identified classified and characterized 10 HML-10 sequences in chromosomes 1, 6, 16, 19 and Y and showed they have the Rec sequence. Five of them have both LTRs, 4 have only 3'-LTR and 1 is without LTRs. Age estimation analysis indicated that they inserted into the genome >25 Mya ago. Five HML-10s conserved the primer binding site region recognizing the Lys tRNA. Translational analysis showed that all proviruses have multiple stop codons that preclude their production of functional proteins. Phylogenetic analyses were performed with complete DNA sequences as well as with the Pol amino acid sequences and will be presented. Precise knowledge of all HML-10 sequences will allow further investigation of their physiological and pathological roles.

P10.5

Could nucleoporins be involved in susceptibility to endometriosis?

M. Cipollini[1], L. Pellè[1], G. Figlioli[1], O. Melaiu[1], E. Barone[1], C. De

Santi[1], E. Paolicchi[1], F. Bambi[2], S. Ermini[2], R. Barale[1], S. Luisi[3], P. Viganò[4], F. Petraglia[3], F. Gemignani[1], S. Landi[1]
[1]Department of Biology, University of Pisa, [2]Blood Centre, University Hospital A. Meyer, Florence, [3]Department of Molecular Medicine and Development, University of Siena, [4]San Raffaele Hospital, Milan

Endometriosis is a debilitating disease affecting 10–15% of reproductive aged women. Nucleolar channel system (NCSs) are small organelles in the nuclei of endometrial epithelial cells appearing transiently, in the mid luteal phase. NCS is composed by various nucleoporins, it seems involved in the endometrial preparation for attachment and implantation of the embryo, but its function remains elusive. In a previous study, we detected a polymorphism within the nucleoporin NUP210 3'UTR with functional activity. In silico analyses showed that the expression of NUP210 could be regulated by miR-125a and miR-125b, two miRNAs up-regulated in ectopic endometrial samples in comparison to the eutopic. In the present work we show that miR-125a affects the expression of a reporter gene chimerized with NUP210 3'UTR and this regulation is genotype-dependent. In addition, we show that this polymorphism is associated with the risk of endometriosis following a case-control association study on 162 cases and 557 controls (rare homozygotes and heterozygotes have a statistically significant increased risk). Overall, these results suggest a link between nucleoporin regulation, NCS, and endometriosis.

P10.6
Modern alpine populations have no maternal lineages related to the Tyrolean Iceman

V .Coia[1], G. Cipollini[1], C. Battaggia[2], P. Anagnostou[2], G. Destro Bisol[2,3], F. Maixner[1], A. Zink[1]
[1]Accademia Europea di Bolzano (EURAC-research), Istituto per le mummie e l'Iceman, Bolzano, Italy, [2]Dipartimento Biologia Ambientale, Università La Sapienza, Roma, Italy, [3]Istituto Italiano di Antropologia, Roma, Italy

After its discovery in the Italian part of the Ötztal Alps in the 90s, numerous archaeological, biochemical and genetic studies have been focused on the mummified body of the Iceman, an individual who lived in the south ridge of the Alps during the Copper Age (~ 5,300 y.a). Recent study on the complete genome of the mitochondrial DNA showed that the Iceman belonged to a branch of haplogroup K1 named K1f, that has not yet been found in extant populations. These results suggest that this lineage might be extinct or very rare. However, the limit of this study was the scarcity of data from European modern populations, especially from the Alpine region of interest. We analyzed the complete mtDNA of 42 K samples, selected from ~850 alpine individuals, in order to increase the comparative data from this key zone and verify the possible presence of the Iceman´s mtDNA lineage. Preliminary results show that no sequence closely related to K1f haplogroup are present in the analyzed samples, which were rather assigned to different European K haplogroups, such as K1a, K1b, K1c, K1e. Analysis of genetic data are ongoing, in order to trace an updated phylogenetic tree of haplogroup K in Europe.

P10.7
Genetic diversity in Amazonian indigenous people from Peru': data from Y-chromosome and mitochondrial DNA

T. Di Corcia[1], O. Maggiulli[1], F. Iuliano[1], C. Martinez-Labarga, T. J. Davila[2], C. Sanchez Mellado[2], O. Rickards[1]
[1]Department of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica n. 1, 00173 Rome, Italy. E-mail: tulliadc84@gmail.com; rickards@uniroma2.it; martine@uniroma2.it, [2]University Alas Peruanas, Av. San Felipe 1109. Jesús Maria, Lima, Peru'

The Asiatic origin of Native Americans is today widely acepted, but the migration route of the first Americans into the continent is not completely understood. The Amazon rainforest covers about 60% of the territory of Peru, but only a small number of indigenous Amazonian populations have been studied. Some archaeological remains have been used to hypotize an Amazonian origin of the first human cultures settled in the peruvian Andes, but other claim a Mesoamerican source population or an Ecuadorian one. We report 160 mitochondrial DNA hypervariable segment sequences of Amazonian Native people from the environs of Pucallpa and Iquitos . We compare our data with others data set from the northern regions of South America, and from Central America to establish which is the best model to explain the peopling of the area. Our preliminary results show an appreciable mitochondrial variability in the Amazon area and the typical haplogroups found in all Native people, sharing some haplotypes also with Andean populations. We are also processing 78 Y-STRs to provide the male contribution in order to get a deeper understanding of the biological history of the Peruvian Native Americans.

P10.8
Novel genome-wide association study-based candidate *loci* for differentiated thyroid cancer risk

G. Figlioli[1], A. Köhler[2], B. Chen[2], R. Elisei[3], C. Romei[3], M. Cipollini[1], A. Cristaudo[3], F. Bambi[4], E. Paolicchi[1], P. Hoffmann[5], S. Herms[5], M. Kalemba[6], D. Kula[6], S. Pastor[7], R. Marcos[7], A. Velázquez[7], B. Jarząb[6], S. Landi[1], K. Hemminki[2,8], A. Försti[2,8], F. Gemignani[1]
[1]Dept of Biology, Univ. of Pisa, Pisa, Italy, [2]Molecular Genetic Epidemiology, DKFZ, Heidelberg, Germany, [3]Dept of Endocrinology and Metabolism, Univ. of Pisa, Pisa, Italy, [4]Blood Centre, Azienda Ospedaliero Universitaria A. Meyer, Firenze, Italy, [5]Dept of Genomics, Life and Brain Center, Univ. of Bonn, Bonn, Germany, [6]Dept of Nuclear Medicine and Endocrine Oncology, Institute of Oncology, Gliwice, Poland, [7]Dept de Genètica i de Microbiologia, Univ. Autònoma de Barcelona, Barcelona, Spain, [8]Center for Primary Health Care Research, Lund University, Malmö, Sweden

Genome-wide association studies (GWASs) on differentiated thyroid cancer (DTC) identified robust associations with 9q22.33, 14q13.3 and 2q35. Our recent GWAS suggested additional *loci* specific for the Italian population. To identify new DTC risk variants, we analyzed 45 SNPs selected from our GWAS first in an Italian population and then in Polish and Spanish cohorts. The combined analysis of the GWAS and the Italian replication study provided strong evidence of association with *BATF* at 14q24.3 (p-*value*=4.3×10^{-7}) and *DHX35* at 20q11.22-q12 (p-*value*=2.13×10^{-8}). A borderline association was also found for *ARSB* at 5q14.1 (p-*value*=8.54×10^{-6}) and *SPATA13* at 13q12.12 (p-*value*=3.25×10^{-6}). Only the associations between *BATF* and *DHX35* and DTC risk were replicated in Polish and the Spanish populations (All studies combined, p-*value*=9.30×10^{-7} and p-*value*=1.34×10^{-8}, respectively). *In silico* analysis suggested that these regions may affect important regulatory mechanisms. Further studies are warranted to identify the specific variants responsible for the observed associations and to validate functional predictions.

P10.9
Ancient human migrations from Arabia to Eastern Africa: new clues from R0a mitogenomes

F. Gandini[1], A. Achilli[2], M. Pala[3], M. Bodner[4], S. Brandini[1], A. Salas[5], R. Scozzari[6], A. Coppa[7], F. Cruciani[6], W. Parson[4,8], O. Semino[1], M. Richards[3], A. Torroni[1], A. Olivieri[1]

[1]Dip. di Biologia e Biotecnologie, Univ. di Pavia, Pavia, Italy, [2]Dip. di Chimica, Biologia e Biotecnologie, Univ. di Perugia, Perugia, Italy, [3]School of Applied Sciences, Univ. of Huddersfield, Queensgate, Huddersfield, UK, [4]Institute of Legal Medicine, Innsbruck Medical Univ., Innsbruck, Austria, [5]Dep. de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Univ. de Santiago de Compostela, Santiago de Compostela, Galicia, Spain, [6]Dip. di Biologia e Biotecnologie "Charles Darwin", Sapienza Univ. di Roma, Rome, Italy, [7]Dip. di Biologia Ambientale, Sapienza Univ. di Roma, Rome, Italy, [8]Penn State Eberly College of Science, Univ. Park, PA, USA

The area around the Bab al-Mandab strait has played a pivotal role in past human migrations, connecting the Horn of Africa with the Arabian Peninsula. Derivatives of the major non-African mitochondrial haplogroups are found at low frequencies in sub-Saharan Africa, mainly as the result of limited and relatively recent migratory events. By analyzing the mtDNA variation of different populations from the Horn of Africa, we observed an unusually high incidence of haplogroup

R0a, which is absent in other areas of the continent and originated in the Arabian Peninsula. In an attempt to reconstruct the migratory and demographic events that led to the spread of this mtDNA haplogroup in Eastern Africa, we analysed 117 published R0a mitogenomes together with 59 newly sequenced ones. The updated worldwide phylogeny shows that ~80% of the African samples cluster in African-specific branches, with age estimates that are compatible with an arrival from southern Arabia around 10,000 years ago.

P10.10
The origin of modern Panamanians: the Y-chromosome perspective

V. Grugni[1], V. Battaglia[1], U.A. Perego[1,2], H. Lancioni[3], M. Tribaldos[4], A. Olivieri[1], I. Cardinali[3], E. Rizzi[5], A. Raveane[1], S.R. Woodward[3,6], J.M. Pascale[4], R. Cooke[7], N. Myres[3,6], A. Torroni[1], J. Motta[4], A. Achilli[3], O. Semino[1]

[1]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy, [2]Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah, USA, [3]Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy, [4]Gorgas Memorial Institute for Health Studies, Panama City, Panama, [5]Institute of Biomedical Technologies, National Research Council (ITB-CNR), Segrate, Milano, Italy, [6]Ancestry, Provo, Utah, USA, [7]Smithsonian Tropical Research Institute, Panama City, Panama

Panama occupies a unique geographic position as the land bridge between North and South America and, according to archaeological evidence, it was continuously occupied by Amerind groups. Today's indigenous groups account for 5.3% of the population and are mainly represented by tribes that settled in Panama from surrounding regions after the autochthonous Natives decimation following the Spanish conquest. However, as demonstrated by a recent study on the mtDNA of the modern Panamanian population (Perego et al., 2012), the ancestral indigenous gene pool was not completely replaced. In particular: (1) at least from maternal side, the Europeans did not contribute significantly to today's genetic composition of Panama; (2) the first settlement of Panama occurred quite rapidly after the initial colonization of the American continent; and (3) the founder ages of the most common lineages support the antiquity of the Pacific coastal route. In this study, we have now tested the same scenario (in modern Panamanians) from the paternal side, taking advantage of the recent improvement in the phylogenetic resolution of Native American Y-chromosome haplogroup Q (Battaglia et al., 2013).

P10.11
Folate metabolism and gene promoter methylation in thymomas of patients affected by Myasthenia Gravis

A. Lopomo[1,2], M. Denaro[1], R. Ricciardi[3,4], A. De Rosa[3], M. Maestri[3], M. Lucchi[4], A. Mussi[4,5], F. Coppedè[1], L. Migliore[1]

[1]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy, [2]Doctoral School in Genetics, Oncology, and Clinical Medicine, University of Siena, Siena, Italy, [3]Department of Neuroscience, Division of Neurology, Pisa University Hospital, Pisa, Italy, [4]Cardiac and Thoracic Department, Division of Thoracic Surgery, Pisa University Hospital, Italy, [5]Department of Surgery, Medical, Molecular, and Critical Area Pathology, University of Pisa, Pisa, Italy

Nowadays growing evidence suggests a contribution of epigenetic processes in promoting cancer and autoimmunity. Myasthenia Gravis is an autoimmune disease mainly mediated by autoantibodies against the nicotinic acetylcholine receptor. 15-20% of the patients present thymoma and there is indication that changes in DNA methylation might contribute to cancer risk and progression. Folate metabolism is necessary for DNA synthesis, repair and methylation. We investigated *MTHFR*, *MTRR*, *TYMS*, and *DNMT3B* folate metabolism gene polymorphisms in MG patients with thymoma and healthy controls. No difference in allele and genotype frequencies were observed, but an increased frequency of the combined *DNMT3B*-579TT/-149CT genotype was found. After gender stratification the effect of *DNMT3B* promoter polymorphisms was restricted to males. Analysis of methylation levels of tumor-related gene promoter showed that *MTHFR* was methylated in all the samples, and h*MLH1* and *CDKN2A* were weakly methylated in few thymoma samples. Present data suggest that *DNMT3B* polymorphisms might contribute to the risk of developing thymoma in male MG patients.

P10.12
Are *MSLN*, *CFB* and *CCNO* cancer genes of mesothelioma?

O. Melaiu[1], E. Barone[1], M. Cipollini[1], G. Figlioli[1], E. Paolicchi[1], L. Pellè[1], C. De Santi[1], L. Mutti[2], A. Cristaudo[3], A. Bonotti[3], R. Foddis[3], F. Gemignani[1], S. Landi[1]

[1]Department of Biology, University of Pisa, Pisa, Italy, [2]Laboratory of Clinical Oncology, Vercelli National Health Trust, Vercelli, Italy, [3]Section of Occupational Medicine, School of Medicine and Surgery, University of Pisa, Pisa, Italy

Genes involved in malignant pleural mesothelioma (MPM) have been poorly characterized and studies have yielded conflicting results. Through a previous research we identified novel candidate genes: *MSLN*, *CCNO* and *CFB*. In order to investigate whether they could play a driving role in MPM, we employed the RNA-interference technique. Transient transfections were performed in a panel of 4 MPM cell lines (NCI-H28, Mero-14, IstMes2, and Ren), and in one mesothelial cell line (Met5A). After *MSLN*, *CCNO* and *CFB* depletion, a series of functional studies were performed to assess cell proliferation rate, cell cycle, apoptosis, cell migration and invasion. *MSLN*-silencing caused decreased proliferation rate and reduced invasive capacity and sphere formation in Mero-14 cells. *MSLN*-siRNA, combined with cisplatin, triggered a marked increase in apoptosis and a decrease in proliferation. *CFB*-siRNA did not cause any significant phenotypic changes. Finally, *CCNO* depletion caused an increased proliferation rate and greater colony formation capacity of Ren cells. These results highlight the extreme heterogeneity of MPM and suggest *MSLN* as a key molecular target for novel gene-based targeted therapies.

P10.13
Genetic analysis of the 16 STR loci for human identification in native Amazonian populations from Perù

F. Messina[1], T. Di Corcia[1], M. Ragazzo[1], C. Sanchez Mellado[2], P. Malaspina[1], B. M. Ciminelli[1], O. Rickards[1], C. Jodice[1]

[1]Department of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica n. 1, 00173 Rome, Italy, [2]University Alas Peruanas, Av. San Felipe 1109. Jesús Maria, Lima, Peru'

Nowadays, human autosomal STRs are widely used for human identification and resolving the forensic cases. Because of Andean mountains in the west and Amazonian Basin in the east, indigenous inhabitants of Peruvian Amazonian region have been considered among the most isolated human groups of the world. Here, we report genetic profiles (16 autosomal STRs) belonging to 142 individuals from Peruvian Amazonian region. We compared indigenous STR data set to other populations from America, Asia and Europe in order to increase the knowledge about autosomal genetic pool of this population and also improve the comprehension of the demic flows in this area. Our preliminary results show a low number of allelic variants and therefore a low variability among these Amazonian communities. Moreover, the relative high frequencies of some private alleles confirm their belonging to native American groups. In conclusion, the genotyping of these indigenous populations allowed us to provide a more accurate description of Amazonian autosomal genetic landscape, further proposing human identification STRs as a useful tool to human migration study.

P10.14
Population analyses of ancient Umbrians through a Next Generation Sequencing approach

A. Modi[1], E. Rizzi[2], H. Lancioni[3], A. Lannino[1], A. Pietrelli[2], L.

Bonomi Ponzi[4], L. Lucentini[3], F. Panara[3], G. De Bellis[2], A. Olivieri[5], S. Ghirotto[6], M. Lari[1], G. Barbujani[6], D. Caramelli[1], A. Achilli[3]
[1]Dipartimento di Biologia Evoluzionistica, Laboratori di Antropologia, Univ. Firenze, Italy, [2]Istituto di Tecnologie Biomediche, Consiglio Nazionale delle Ricerche (ITB-CNR), Segrate (MI), Italy, [3]Dipartimento di Chimica, Biologia e Biotecnologie. Univ. Perugia, Italy, [4]Soprintendenza per i Beni Archeologici dell'Umbria. Perugia, Italy, [5]Dipartimento di Biologia e Boiotecnologie "L. Spallanzani". Univ. Pavia, Italy, [6]Dipartimento di Scienze della Vita e Biotecnologie. Univ. Ferrara, Italy

Since prehistoric times, Umbria has been a central point for the trans-Apennine routes between the Tyrrhenian regions and the Adriatic coast. Unfortunately few data and records are available to understand the population dynamics on ancient Umbria. We aimed to shed light on this issue through a genetic study by investigating the interactions between the ancient Umbrians (*Umbri Plestini*) and other surrounding populations, such as the Etruscans, and by evaluating the contribution to the modern gene pool. We analyzed the ancient DNA of 35 skeletons from Colfiorito necropolis (IX-V sec b.C.) by amplifying the mitochondrial region HVS-I. The target region was sequenced by Next Generation Sequencing (NGS) and, after an *ad-hoc* bioinformatics filtering, the ancient data were compared with 48 modern populations (from Italy and Western Eurasia) and with four Iron Age European populations. In brief, the *Umbri Plestini*showed: high intrapopulation variability and few shared haplotypes both modern and other ancient populations. For some samples the results will be validated by Target Enrichment and NGS of the entire mitogenome in order to depict a more accurate scenario.

P10.15
Charting the most likely geographical origin of maternal and paternal human lineages in mixed Ecuadorian populations

G. Primativo[1], C. Martinez-Labarga[1], MJ. Alvarez-Cubero[3,4], G. Biondi[2], G.F. De Stefano[1], M. Saiz[4], J.A. Lorente[3], O. Rickards[1]
[1]Centro di Antropologia Molecolare per lo studio del DNA antico, Dipartimento di Biologia, Università di Roma "Tor Vergata", Roma, Italia, [2]Dipartimento di Scienze Ambientali, Università dell'Aquila, L'Aquila, Italia, [3]GENYO. Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica. Parque Tecnológico de Ciencias de la Salud (PTS), Granada, España, [4]Laboratorio de Identificación Genética. Departamento de Medicina Legal y Toxicología. Facultad de Medicina, Universidad de Granada, Granada, España

Atlantic slaves trade gave an important contribution to the settlement of the Americas. During the 15th and 16th centuries, millions of African people were brought to the New Continent by Europeans *conquistadores* and when slaves got freedom they moved all around the Continent. Since this event, mixed unions with Native populations or Europeans became more frequent. Traces of these admixtures can be found in the genome of the extant populations. In order to reconstruct the maternal and paternal genetic history of two mixed populations of Ecuador (Rio Cayapas and Viche), both living in the Esmeraldas province, we analyzed Y chromosome and mitochondrial DNA (mtDNA) variation. In particular, to determine the male contribution; we amplified 17 Y-STR loci using the AmpFSTR Yfiler Kit (Applied Biosystems, Forster City, CA, USA). Then, through the High Resolution Melting (HRM) technique, we tested diagnostic SNPs to confirm the haplogroup designation in relation to the haplotype profiles. Maternal lineages were also identified by sequencing of hypervariable segments I and II of mtDNA D-loop region and in some selected samples by the analysis of the entire mtDNA genome.

P10.16
Early human dispersal from Africa: a model-based test of two hypotheses

F. Tassi[1], S. Ghirotto[1], M. Mezzavilla[2], S. Torres Vilaça[3,4], L. De Santi[1], G. Barbujani[1]
[1]Department of Life Sciences and Biotechnologies, University of Ferrara, Italy, [2]Institute for Maternal and Child Health - IRCCS "Burlo Garofolo" - Trieste , Italy and University of Trieste, Italy, [3]Evolutionary Genetics, Leibniz-Institute for Zoo and Wildlife Research, Berlin, Germany, [4]Berlin Center for Genomics in Biodiversity Research, Berlin, Germany

It is unclear whether early modern humans left Africa through a major migration process, dispersing simultaneously over Asia and Europe, or in two main waves, first through the Arab peninsula into Southern Asia, and later through the Near East into Western Asia and Europe. We collected a broad genome-wide SNP dataset in 72 populations to test if single (SD) or multiple (MD) dispersal models can better account for patterns of genome diversity. We found good correlations between geographic and genetic distances, but only insignificant differences between models. We moved to consider the patterns of Linkage Disequilibrium in each population to estimate effective population size, that allows us to assess when population pairs diverged in time. We found a good correlation between LD estimates and geographic distances from Africa, but only when geographic distances were calculated according to a MD model. Then, we estimated different divergence times from Africa for Asians and Europeans. Simulated genetic polymorphism data demonstrate the validity of our conclusions. Our results are in support of MD model into Eurasia, with some populations retaining the signal of a later dispersal.

P10.17
MiR34a oncosuppressor function in paediatric embrional tumors

M. Zollo[1], P. de Antonellis[2], M. Carotenuto[3]
[1]Dip. Medicina Molecolare e Biotecnologie Mediche Università Federico II, Napoli, Italy, [2]CEINGE Biotecnologie Avanzate scarl, Napoli, Italy, [3]CEINGE Biotecnologie Avanzate scarl, Napoli, Italy

Through negative regulation of gene expression, microRNAs (miRNAs) can function as oncosuppressors in cancers, and can themselves show altered expression in various tumor types. Several genes, encoding for proteins involved in proliferation, invasion and apoptosis, are known to be direct miR-34a targets. Here, we used proteomics to screen for early targets of miR-34a in neuroblastoma (NBL), a childhood cancer that originates from precursor cells of the sympathetic nervous system. Thanks to these analysis out to 2,082 proteins, we found 7 new gene products (ALG13, TIMM13, TGM2, ABCF2, CTCF, Ki67 and LYAR) that correlated with worse clinical outcome. These proteins affect signaling pathways regulating cell proliferation and cell cycle enhancing tumor progression (TGF-β, WNT, MAPK and FAK).We then investigates its role in medulloblastoma tumors, which arise from an early impairment of developmental processes in the cerebellum. We study how miR34a regulate Notch in a subset of MB cells that have stem-cell-like properties and can promote tumor growth. We hypothesized that miRNAs targeting the Notch pathway can regulate these phenomena, and its use in anti-cancer therapies.

O10.1
A case-control association study suggests a role for *CYP2E1* in the susceptibility to thyroid carcinoma

L. Pellè[1], M. Cipollini[1], G. Figlioli[1], O. Melaiu[1], E. Barone[1], C. De Santi[1], E. Paolicchi[1], R. Foddis[2], A. Cristaudo[2], A. Bonotti[2], F. Bambi[3], S. Ermini[3], C. Romei[3], R. Elisei[3], G. Pellegrini[4], R. Barale[1], S. Landi[1], F. Gemignani[1]
[1]Department of Biology, University of Pisa, Pisa, Italy, [2]Section of Occupational Medicine, School of Medicine and Surgery, University of Pisa, Pisa, Italy, [3]Department of Endocrinology and Metabolism, Orthopedics and Traumatology, Occupational Medicine, University of Pisa, Pisa, Italy, [3]Blood Centre of University Hospital of Meyer, Florence, Italy, [4]Operative Unit of Laboratory of Clinical Chemistry Analyses, University Hospital of Cisanello, Pisa, Italy

Thyroid carcinoma results from a complex interaction between genetic and environmental factors. Acrylamide (AA) is carcinogenic in rodents inducing thyroid carcinomas of differentiated histotype (DTC). AA is classified by IARC as "probable carcinogen" and it is found in foods,

following Maillard's reaction. AA is biotransformed *in vivo*mainly by cytochrome 2E1 (CYP2E1) to its epoxide glycidamide (GA), which is hypothesized to be the ultimate carcinogen. Four SNPs within *CYP2E1* were selected (one at the promoter region, three spanning introns 5-8) for a case-control association study. A preliminary analysis of 350 cases and 350 controls showed an association between one SNP within intron 8 (SNP8) and risk of DTC. Thus, the analysis was extended to a replication set consisting of 2429 controls and 767 cases confirming the association: OR for c1/c2 and c2/c2 genotypes versus c1/c1 genotype were 1.24 (95% CI 1.03-1.48) and 1.56 (95% CI 1.06-2.30), with p-value 0.02 and 0.02, respectively. The current hypothesis is that one SNP within *CYP2E1* could modulate the activity of the enzyme, thereby affecting the metabolism of AA. Actually, other *in vitro* data support this hypothesis.

O10.2
Exploring genetic variability of genes involved in nutrition and thermoregulation processes in european populations

A. Quagliariello[1,2], M. Sazzini[1,2], L. Pagani[3], A. Cherubini[1,2], S. De Fanti[1,2], C. Giuliani[1,2], D. Luiselli[1,2]
[1]Laboratory of Molecular Anthropology, [2]Centre for Genome Biology Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Bologna, Italy, [3]Division of Biological Anthropology, University of Cambridge, UK

Environmental and cultural changes, such as those affecting dietary habits, are known to have substantially affected populations' allele frequencies over human evolutionary history, triggering several adaptive processes. In some cases, especially since the Neolithic transition, they were established so rapidly to prevent adequate genetic adaptation to new nutritional/ecological conditions, thus potentially leading to various common chronic diseases. To detect genetic signatures of some of these evolutionary events and taking advantage from the 1000Genomes project dataset, we have analysed patterns of variation at a set of genes involved in nutritional and thermogenesis processes. Our preliminary results indicate *PRDM16*, a gene involved in the development of brown and beige adipocytes, as the most polymorphic locus within Europe. For instance, clusters and FST analyses pointed out completely different patterns between northern and southern European populations, suggesting the plausible action of climatic selective pressures on this gene. This study was conducted within the framework of the EPIC project (PRIN2012).

O10.3
Whole genome sequencing of 3,514 individuals from the founder population of Sardinia

C. Sidore[1,2,3], F. Busonero[1,2,4], A. Maschio[1,2,4], M. Zoledziewska[1], A. Mulas[1,3], E. Porcu[1,2,3], G. Pistis[1,2,3], M. Steri[1], F. Danjou[1], F. Deidda[1], C. W. Chiang[7], M. Pitzalis[1], C. Jones[5], A. Angius[1,5], H. M. Kang[2], J. Novembre[8], S. Sanna[1], D. Schlessinger[6], G. R. Abecasis[1], F. Cucca[1,3]
[1]Istituto di Ricerca Genetica e Biomedica, CNR, Monserrato, Cagliari, Italy, [2]Center for Statistical Genetics, Ann Arbor, University of Michigan, MI, USA, [3]Università degli Studi di Sassari, Sassari, Italy, [4]University of Michigan, DNA Sequencing Core, Ann Arbor, MI, USA, [5]Center for Advanced Studies, Research, and Development in Sardinia (CRS4), AGCT Program, Parco Scientifico e tecnologico della Sardegna, Pula, Italy, [6]Laboratory of Genetics, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA, [7]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA, [8]Department of Human Genetics, University of Chicago, IL, USA

Recently, the next generation sequencing in worldwide populations, i.e. the 1000Genomes project, increased the resolution of genetic analyses and described the variation shared among populations. However the variation in isolated populations may be under represented due to their large genetic distance. We investigated the Sardinian variation with whole genome sequencing (coverage ~4x) of 3,514 individuals, identified >23M single nucleotide polymorphisms (SNPs) and generated a reference panel for imputation improving the imputation accuracy of rare variants (MAF 0.5-5%, r2 with genotypes=0.9). We will show how isolation affects enrichment of functional variants very rare or absent elsewhere, haplotype length, genetic differentiation with Europeans and Y chromosome phylogeny. We studied the genetics of >500 quantitative traits (including lipid levels, haematological, immunological and anthropometric measures) by imputing the 23M SNPs in 6,602 individuals from the SardiNIA cohort, living in the Ogliastra region. We found numerous novel associations that illustrate how sequencing this founder population helps detect traits-associated variants that could have been missed otherwise.

O10.4
Y-chromosome and mtDNA diversity in the context of Eurasian language diversity

R. R. Susca[1], S. Sarno[2], F. Tassi[1], S. Ghirotto[1], N. Russo[1], C. Guardiano[3], D. Luiselli[2], D. Pettener[2], G. Longobardi[4], G. Barbujani[1]
[1]Dept Life Sciences and Biotechnologies, University of Ferrara, Italy, [2]Dept Biological, Geological and Environmental Sciences, University of Bologna, Italy, [3]Dept Communication and Economics, University of Modena and Reggio Emilia, Italy, [4]Dept Language and Linguistic Science, University of York, UK

The central goal of the ERC advanced grant project LanGeLin (LANguage-GEne LINeages) is to investigate the relationship between genetic and linguistic diversity, the latter inferred from structural language features, rather than from the vocabulary. Y chromosome (Y-chr) and mtDNA provide complementary information and allow one to investigate the different migrational histories of males and females, and their impact over the global language-gene relationships. We assembled two datasets including 27 populations for which both mtDNA/Y-chr and linguistic data were available. We calculated and compared phylogenetic trees and Mantel's correlations between genetic, linguistic and geographical distances starting from three matrices: d_{GEN} based on F_{ST} (genetic distances); d_{SYN} based on syntactic features (linguistic distances); and d_{GEO} based on geographical distance between pairs of populations. Both similarities and differences were evident between patterns of genetic and linguistic variation, casting light on both the genealogical ties between populations, and the mechanisms of language change.

O10.5
Phylogenetic refinement and SNP-based dating of human MSY haplogroup E

B. Trombetta[1], E. D'Atanasio[1], A. Massaia[1], A. Coppa[2], G. Russo[3], A. Novelletto[4], R. Scozzari[1], F. Cruciani[1]
[1]Sapienza Università di Roma, Dipartimento di Biologia e Biotecnologie "Cherles Darwin", Rome, Italy, [2]Sapienza Università di Roma, Dipartimento di Biologia Ambientale, Rome, Italy, [3]Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Rome, Italy, [4]Università Tor Vergata Dipartimento di Biologia

Increasing the resolution of the human Y chromosome tree has led to a better temporal and spatial understanding of past population dynamics. Haplogroup E is the most represented Y chromosome clade within Africa, and it is also found at relatively high frequencies across West Asia and Europe. To increase the level of phylogenetic resolution of this clade, we characterized by high-coverage (50x) next generation sequencing a set of 18 haplogroup E chromosomes. We identified a total of 559 variant positions, 76% of which novel. We constructed a maximum-parsimony Y tree and estimated the most recent common ancestor for all the nodes using a SNP-based approach. The phylogeny showed several novel features compared to the previous topology: the relative length of branches was drastically modified and the associated node ages changed. A subset of the 559 variants was used to genotype sixty additional Y-chromosomes belonging to the E-M35 sub-haplogroup. By this analysis, we resolved all previously known polytomies and assigned all the E-M35* chromosomes to different new monophyletic clades, thus increasing the discriminative power of the haplogroup for use in human evolution and forensics.

11 - Genetic of Microorganisms

P11.1

Analysis of intestinal microbial biodiversity in newborns exposed to intrapartum antibiotic prophylaxis by means of a massive sequencing approach

S. De Fanti[1], A. Quagliariello[1], I. Aloisio[2], G. Mazzola[2], M. Sazzini[1], L.T. Corvaglia[3], D. Luiselli[1], D. Di Gioia[2]

*[1]Laboratory of Molecular Anthropology * Centre for Genome Biology Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Bologna, Italy, [2]Department of Agricultural Sciences, University of Bologna, viale Fanin 42, 40127 Bologna, Italy, [3]Neonatal Intensive Care Unit, S. Orsola Malpighi Hospital, Via Massarenti 11, 40138 Bologna, Italy*

Next-Generation DNA sequencing data from the human intestinal microbiome provides new instruments to explore the correlation between changes in microbiome composition and the host physiologic status. Some studies show that newborns have a highly unstable microbiome composition, but few information are available in literature on the potential effect due to intrapartum antibiotic prophylaxis (IAP) administrated to the mothers positive to the infection by group B *Streptococcus*, which affects around 10% of pregnant woman. According to this, the aim of the project is to evaluate the main effects of the mentioned IAP on microbiome composition of newborns at seven days after birth, by a massive parallel sequencing of seven different regions of 16S gene. The analysis was performed with Ion PGM platform, taking advantage from "Ion 16s Metagenomics Sequencing Technology Access Program" (*LifeTechnologies*). Our first results show an evident difference in the microbial composition of treated newborns, resulting in a general decrease of microbial biodiversity and an overrepresentation of Proteobacteria, suggesting that these infants are potentially more exposed to gastrointestinal diseases.

P11.2

Genetic, physiological and molecular characterization of *MPV17/SYM1* mutations in *Saccharomyces cerevisiae*

M. Gilberti, C. Donnini, I. Ferrero, C. Dallabona

Department of Life Sciences, University of Parma, Parma, Italy

MPV17 is an intriguing gene necessary for the maintenance of mitochondrial DNA (mtDNA) in human, mutation of which leads to a peculiar form of hepatocerebral mtDNA depletion syndrome (MDS) that are genetic disorders characterized by a severe, tissue-specific decrease of mtDNA copy number. In spite that Mpv17 mutations are a prominent cause of MDS in humans its function remains a baffling and challenging issue. Originally considered as a peroxisomal membrane protein, it was later demonstrated that Mpv17 is localized to the inner mitochondrial membrane, as also previously demonstrated for the yeast orthologue Sym1, identified as a heat shock protein with a role in metabolism and/or tolerance to ethanol. Studies in yeast have shed some light on the role of Sym1, but the specific function remains elusive. In order to define the molecular basis and clarify the pathological role of MPV17 variants associated with the human disorder, we have taken advantage of *S. cerevisiae* as a model system. We studied the effect of 7 pathological mutations on the cell physiology, in particular on mitochondrial function. Furthermore we determined their molecular effects on the protein.

P11.3

Systems biology analysis of *Helicobacter pylori* heat-shock response: dissecting complex multi-layered regulatory strategies of gene expression

D. Roncarati, E. Fiore, T. Brignoli, D. Cecchi, A. Danielli, V. Scarlato

Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Bologna, ITALY

Bacteria respond to heat stress by reprogramming the expression of specific genes coding for chaperones and heat-shock proteins (HSPs). Accordingly, complex strategies have evolved to regulate HSPs expression in different microbial species. *H. pylori* controls the expression of chaperone-coding operons with a circuit comprising two auto-regulated repressors, HrcA and HspR. This circuit is wired in an incoherent type 2 FFL motif, responsible for the rapid induction of *groE*promoter upon heat shock. Circuits of this kind have a crucial interest for systems biology, and applicative potential in synthetic biology. We characterize the circuit by verifying with fluorescent markers the output dynamics of a heterologous form of the circuit in *E. coli*. Moreover, to address the regulative interactions of HspR and HrcA with other genes, and to gain a comprehensive picture of *H. pylori* heat shock regulation, we implement a integrative "omics"-strategy that combines a systemic ChIP-seq approach, to spot new targets for the two regulators, with the heat-shock transcriptome and proteome. This study will shed light on how this pathogen explore complex gene expression control in response to stress.

P11.4

Investigating new pathoadaptive mutations in *Shigella* and EIEC strains given rise to the interaction with host cells

A. Leuzzi[1], M.L. Di Martino[1,2], M. Casalino[1], M. Grossi[1], B. Colonna[1], G. Prosseda[1]

[1]Istituto Pasteur-Fondazione Cenci Bolognetti , Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma, Via dei Sardi 70, 00185 Roma, Italy, [2]Dipartimento di Scienze, Università Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy

Shigella is a highly adapted human pathogen, mainly found in the developing world and causing a severe and extremely contagoius enteric syndrome, Shigellosis, which may be fatal in children. Enteroinvasive *E. coli* (EIEC) are a group of *E. coli* which share with *Shigella*the same invasive process. The highly sophisticated infectious strategy of *Shigella*and EIEC banks on the capacity to invade, disrupt, and cause inflammatory destruction of the intestinal epithelial barrier. Besides the extensive gain of virulence factors, mainly located on a large virulence plasmid (pINV), the evolution of *Shigella*has been shaped also by a loss of gene functions wich may be detrimental to the pathogenic lifestyle. These modifications, referred to as pathoadaptive mutations, has improved the fitness of *Shigella*within its only prey, the human host .Our work is focused on the role of two new potential pathoadaptative mutations in *Shigella*and EIEC strains as loss of curli fibers and loss of lactose catabolic pathways.

P11.5

Abiotic signal transduction pathways in the classical eukaryotic model system: yeast

M. Proietto, L. De Angelis, A. Visca, MM. Bianchi, P. Ballario

Dept Biologia e Biotecnologie Charles Darwin, Sapienza Università di Roma, Roma, Italia

Our research is dedicated to signal transduction in fungi. The signals we take in consideration are basal environmental stimuli as light, oxygen and glucose and their cross-talking. Light induces several responses notonly in *Neurospora crassa* but also in the hypogeous fungus *Tubermelanosporum*. The photoreceptor involved is the heterodimer WC-1/WC-2 andIn particular the sensor LOV (Light Oxygen Voltage) domain present in WC-1subunit. The photochemical characteristic and the potential utilization of theLOV domain as a molecular switch for the construction of new molecules,suitable as tools for biotechnology and optogenetics, will be investigated.Glucose transport in yeasts is regulated by environmental glucoseconcentration. In the respiratory yeasts, like *Kluyveromyces lactis*, carbonmetabolism is strongly dependent on oxygen availability that influences theequilibrium between respiration and fermentation and lipid metabolism. Theexpression of

the low-affinity glucose transporter (Rag1) is also affected byoxygen: the correlations between the glucose signaling cascade and hypoxiawill be explored in the regulation of the glucose transporter gene *RAG1* in K.lactis.

P11.6
Characterization of gut microbiome and mycobiome in patients with Rett syndrome

F. Strati[1,2], D. Renzi[3], A. Calabrò[3], D. Cavalieri[1]and C. De Filippo[4]
[1]Department of Computational Biology, Comparative Genomics Unit, Fondazione Edmund Mach, San Michele all'Adige, Italy, [2]Centre for Integrative Biology, University of Trento, Trento, Italy, [3]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy, [4]Department of Food Quality and Nutrition, Nutrition and Nutrigenomics Unit, Fondazione Edmund Mach, San Michele all'Adige, Italy

Rett syndrome (RTT) is a neurodegenerative disorder caused by a mutation in MECP2. To date, the mechanism that trigger the disease have yet to be explained. Gut microbiome controls CNS activities through endocrine, metabolic and immune pathways and yeast infections shift IDO's activity leading to a reduction of kineurenine that act as a neuroprotective agent. In addition, kineurenine is affected by the immune system, dysfunction of which has been implicated in the etiology of ASD. The hypothesis we want to test is if alterations in microbiota-mediated immunomodulation are reflected in alterations in microbiota-mediated neuromodulation. We characterized the gut microbiome/mycobiome of RTT patients through culture based and metagenomics approaches. Stool samples from 50 RTT patients and 30 HS have been collected. DNA has been extracted and submitted to metagenomic analysis in order to analyze bacterial and fungal communities' structure. We discovered *C. albicans* as significantly more represented in heathy controls with respect to RTT patients. On the contrary, the highly emerging potentially invasive species, *Trichosporon spp.* and *S. cerevisiae* have been isolated solely in RTT patients.

O11.1
MeDuSa: a multi-draft based scaffolder

E. Bosi[1], B. Donati[2,3], S. Brunetti[4], M. Galardini[5], M. F. Sagot[3], P. L. Crescenzi[2], R. Fani[1], P. Liò[6], M. Fondi[1]
[1]Dept Biology, Florence Univ., Florence, Italy, [2]Dept Systems and Computer Science, Florence Univ., Florence, Italy, [3]Dept Biometry and Evolutionary Biology, Claude Bernard Univ., Lyon, France, [4]Dept Information Engineering and Mathematics, Siena Univ., Siena, Italy, [5]European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Cambridge, UK, [6]Dept Computer Science, Cambridge, UK

Completing the genome sequence of an organism is an important task in comparative, functional and structural genomics. This however remains a challenging issue from both a computational and an experimental point of view. Genome scaffolding (i.e. the process of ordering and orientating contigs) of de novo assemblies usually represents the first step in most genome finishing pipelines. We present MEDUSA (Multi-Draft based Scaffolder), a computational software for ordering and orientating contigs of de novo assembled genomes. MEDUSA exploits information obtained from a set of draft genomes from closely related organisms and modelled into a graph for analysis. As such, it does not require either the presence of a closed reference genome or the use of paired end reads for gap closure. This makes usability an interesting feature of the software. Moreover, our experiments show that MEDUSA is highly accurate and outperforms traditional scaffolders based on paired-end reads.

O11.2
Towards the characterization of the role of YnfB protein in *E.coli*

R. Campilongo, M. L. Di Martino, A. Leuzzi, B. Colonna, G. Prosseda
Istituto Pasteur Fondazione Cenci Bolognetti- Dept. Biology and Biotechnology, Sapienza University of Rome, Rome, Italy

Shigella, a gram negative bacteria, is the causative agent of bacillary dysentery.The virulent phenotype of Shigella is due to the presence of a large virulence plasmid that contains genes whose expression is essential for the infection process. The genomes of *Shigella*and *E. coli*, its commensal ancenstor, are colinear and highly homologous. The genetic differences are the result of an evolutionary process that led to the acquisition of virulence genes and loss of genes that are contrary to the fitness of the bacterium and the disease process. We have focused our attention on*speG*gene mutation patoadattative, which encodes spermidine acetyltransferase catalyzing the conversion of spermidine into the physiological inert acetylspermidine. We show that the *speG*gene is inactive in *Shigella*and that the consequent accumulation of spermidine strongly favours the survival of the pathogen under oxidative stress conditions, as well as within the macrophages it invades during infection. The *speG*gene is inactive in *Shigella*and it has been shown that the consequent accumulation of spermidine strongly favours the survival of the pathogen under oxidative stress conditions. *speG*gene is located within the *ynfB-speG*locus. The *ynfB*gene coding for a protein whose function is not yet known. The aim of my work is to characterize the protein YnfB, trying to understand its function and verifying its alleged involvement in a metabolic pathway of polyamines.

O11.3
Helicobacter pylori acid acclimation: *ménage à trois* at the complex *arsR* promoter

D. Roncarati, S. Pelliciari, A. Vannini, L. Valzania, L. Mazzei, B. Zambelli, S. Ciurli, V. Scarlato, A. Danielli
Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Bologna, ITALY

The essential auto-regulated ArsR response regulator controls transcription of many *H. pylori*pH-dependent genes. The metal-dependent regulators Fur and NikR are also important for regulation of several pH-responsive genes. Accordingly, *fur* and *nikR* mutants are impaired in their acid tolerance and in rodent infection models. Here, we show that the these regulators are engaged in a complex regulatory cascade controlling the *arsR* promoter. Fur binds with different affinities three operator elements, thereby repressing *arsR* transcription in an iron-dependent fashion. This involves the bending of DNA, induced by the interaction of Fur with two adjacent operators encasing the transcriptional start site. In the presence of Ni^{2+}, NikR binds to this region and antagonizes the binding of Fur, releasing the *arsR* operator needed for auto-repression by ArsR. This antirepression mechanism guarantees the acid-inducible expression of the ArsR-dependent urease cistrons only when the intracellular Ni^{2+}levels are sufficiently high to cofactor the urease enzyme. Most importantly, it allows unifying metal ion homeostasis and acid acclimation in a comprehensive and mechanistically coherent model.

O11.4
High yield production of avenanthramide analogous endowed with antioxidant properties

A. Moglia[1], L. Goitre[2], S. Gianoglio[1], E. Baldini[3], A. Genre[4], L. Trabalzini[3], J. Beekwilder[5], S.F. Retta[2]
[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Largo Paolo Braccini 2,10095 Grugliasco (Italy), [2]Dipartimento di Scienze Cliniche e Biologiche, Università degli Studi di Torino, Regione Gonzole 10, 10043 Orbassano (TO), [3]Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Via Fiorentina 1,53100 Siena, [4]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Viale Mattioli 25, 10125 Torino, [5]Plant Research International, P.O. Box 16, 6700 AA Wageningen (The Netherlands)

A novel fermentation system for the heterologous production of phenolic amides, N-(E)-*p*-coumaroyl-3-hydroxyanthranilic acid (Yeast avenanthramide I, Yav I) and caffeoyl-3-hydroxyanthranilic acid (Yav II), was set up by engineering a *S. cerevisiae* strain with two genes (4cl-2 from tobacco and hct from globe artichoke). These novel compounds exhibited a strong structural similarity with oat avenanthramides, a group

of natural antioxidants. The production of yeast avenanthramides reached a final yield of 120 mg/l and 22 mg/l of Yav I and Yav II. To examine the biological relevance of Yav we tested their anti-oxidant properties in MEF and the HeLa cell lines by analyzing their effects on master regulators of cell antioxidant responses, SOD2 and its transcriptional regulator FoxO1. Real Time PCR and Western Blot analysis suggested that yeast avenanthramides positively regulate the anti-oxidant defense mechanism through the up-regulation of FoxO1 and SOD2 expression level. Furthermore, we demonstrated that YAv may exert an anti-proliferative effect by facilitating the down-regulation of Cyclin D1 required from cell transition from proliferative growth to quiescence.

O11.5
Cell cycle regulation by CtrA in *Sinorhizobium meliloti*

F. Pini[1], N.J. De Nisco[2], L. Ferri[3], M. Brilli[4], J. Penterman[2], M. Bazzicalupo[5], A. Mengoni[5], A. Fioravanti[1], P.H. Viollier[6], G.C. Walker[2], E.G. Biondi[1]

[1]Interdisciplinary Research Institute, CNRS, Villeneuve d'Ascq, France, [2]Department of Biology, MIT, Cambridge, USA, [3]Meyer Children Hospital, University of Florence, Firenze, Italy, [4]Fondazione Edmund Mach/CRI, Functional genomics, San Michele all'Adige, Italy, [5]Department of Biology, University of Florence, Firenze, Italy, [6]Dept. Microbiology & Molecular Medicine, University of Geneva, Genève, Switzerland

Sinorhizobium meliloti infects the roots of the legume *Medicago sativa*, whereupon it undergoes an alteration of its cell cycle differentiating into nitrogen-fixing, elongated and polyploid bacteroid. Preliminary data indicate that the architecture of cell cycle in *S. meliloti* is similar to *Caulobacter crescentus* with CtrA as the principal cell cycle regulator. Here we show that depletion of the *S. meliloti* CtrA led to a lethal phenotype with enlarged cells and massive DNA replication. We investigated the CtrA-controlled genes by ChIP-Seq analysis, microarrays and lacZ fusions, demonstrating that CtrA is controlling key genes involved in chemotaxis, motility, cell division and cell cycle progression. We further showed using synchronized cells that *S. meliloti* CtrA oscillates during cell cycle, being degraded at the G1-S transition by an RcdA/CpdR/ClpXP-dependent process. In conclusion, the *S. meliloti* CtrA controls crucial cell cycle functions and the downregulation of CtrA phenotype coincides with bacteroid differentiation. Finally, we propose a model of the cell cycle regulation that shows similar logic to *Caulobacter* but a different architecture of the circuit.

12 - Evolution

P12.1
Parallel domestication of common bean in Mesoamerica and Andes

E. Bellucci[1], E. Biagetti[1], M. El Baidouri[2], B. Abernthy[2], A. Ferrarini[3], E. Bitocchi[1], A. Minio[3], M. Delledonne[3], L. Nanni[1], S.A. Jackson[2], R. Papa[1]
[1]Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona (Italy), [2]Center for Applied Genetic Technologies, University of Georgia, Riverbend Road 111, 30602 Athens (USA), [3]Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona (Italy)

Domestication is a fundamental evolutionary process described as an increasing co-dependence between plants or animals and humans that has resulted in genetic modifications due to human selection. Common bean (*Phaseolus vulgaris* L.) presents a unique evolutionary history among most crops as it characterized by the presence of two main geographically and genetically distinct gene pools, Mesoamerica and Andes, where, at least two independent domestication events occurred. This intriguing scenario makes *P. vulgaris* a useful model to study the domestication process, that can be investigated as a type of replicated experiment. Starting from a study where RNA-Seq approach was used to sequence the transcriptome of a set of wild and domesticated genotypes from Mesoamerica, we applied the same approach to samples of Andean gene pool. Here we report the results of a comparison between genomic and transcriptomic data from the two gene pools of the species.

P12.2
Phylogenetic analysis of β-glucuronidases genes in Angiosperms

M. Ronchini[1], M. Zilio[1], L. Bruno[2], B. Bitonti[2], T. Corinti[1], I. Vanetti[1], P. Gerola[1], G. Binelli[1]
[1]Dept Theoretical & Applied Sciences, Univ. of Insubria, Varese, Italy - [2]Dept Biology, Ecology and Earth Sciences, Univ. of Calabria, Arcavacata di Rende, Italy

This work is aimed at investigating the evolution of β-glucuronidases in plants. β-glucuronidases (GUS) are glicosyl-hydrolases (GHs) that catalyse the hydrolysis of the glycosidic bond between glucuronic acid and other carbohydrates or molecules different from sugars, termed aglycones. GUSs have been identified in all living organisms and, according to their amino acid sequence, classified into three families: GH1 GH2 and GH79. Only the proteins of the GH79 family have been demonstrated to be present in plants, where they are widely distributed. The first sequenced *GUS* gene was in *Scutellaria baicalensis*, whilst in *Arabidopsis thaliana*three *GUSs* have been identified. As model species we used tobacco, where at least three different *GUS* cDNA sequences were obtained, and compared with those available from GenBank in a large number of Angiosperms and also from Gymnosperms, seedless vascular plants and Bryophytes. These data were used for phylogenetic analysis and the possible inferences drawn by the analysis of the resulting tree are discussed. The recently published tobacco genome will also allow us to better understand the evolutionary dynamics of this class of genes.

P12.3
New insights into the characterization of the ancestral centromere on human chromosome 2

G. Chiatante, G. Giannuzzi, M. Ventura
Department of Biology, University of Bari, Bari, Italy

Human chromosome 2 is the product of a head-to-head fusion of two acrocentric ancestral chromosomes, IIp and IIq, which are still separated in great apes. The dicentric chromosome originated from the fusion reached stability by inactivating one centromere corresponding to the IIq, through the loss of alphoid DNA, via poorly understood mechanisms. Unlike the fusion point, the ancestral centromere mapping at 2q21.1-2q21.2 has been barely investigated. Here we performed comparative *in silico* and molecular analyses in chimpanzee, gorilla, orangutan and macaque genomes to shed light on the genomic organization of the 2.1Mb region encompassing the ancestral centromere. This approach allowed us to track precisely its evolutionary history and to investigate the corresponding pericentromeric region, whose assembly is complicated by segmental duplications. Interestingly we found relicts of various repetitive elements that ancestrally surrounded the active centromere; we used them to create a detailed overview of their genome-wide distribution. Finally we proposed a model for the centromere silencing that was likely to be a nonrandom inactivation forced by the genomic structure of the region.

P12.4
Seeing through the skin: dermal light sensitivity provides cryptism in moorish gecko

M. Trapanese[1], D. Fulgione[1], V. Maselli[1], D. Rippa[1], M. Buglione[1], F. Itri[2], B. Avallone[1], D. M. Monti[2], P. Raia[3]
[1]Dept of Biology, Federico II Univ., Naples, Italy, [2]Dept of Chemical Sciences, Federico II Univ., Naples, Italy, [3]Dept of Earth Science, Environment and Resources, Federico II Univ., Naples, Italy

Concealment and camouflage by means of color change is a preeminent deceptive mechanism used by both predators and prey. The Moorish gecko *Tarentola mauritanica*is able to blend into the substrate by matching its level of darkness actively according to the light conditions. Here we examined the early steps of background perception in Moorish gecko responsible for its ability to change colour which lead to its camouflage. We experimentally exclude the involvement of melano-stimulating hormone in skin shading shift. Still, blindfolded individuals change their colour consistently with the background. Surprisingly, individuals with covered flanks were not able to change colour. Accordingly, we found high levels of opsin transcript and protein in the flank region of gecko's trunk. These observations suggest that gecko's skin melanophores are able to activate a process of colour change autonomously. This study yields the first evidence of crypsis mediated by dermal light sensitivity in amniotes.

P12.5
Genetic peculiarities of the wild boar in Italy

H. Lancioni[1], F. Vercillo[1], M.R. Capodiferro[1], I. Cardinali[1], M. Sabino[1], M. Biagetti[2], F. Vincenti[2], C. Sebastiani[2], F. Dionigi[1], L. Iacolina[3], E. Rizzi[4], A. Olivieri[5], U. Sergiacomi[6], M. Scandura[3], A. Torroni[5], B. Ragni[1], A. Achilli[1]
[1]Dept. Chemistry, Biology and Biotechnology, University of Perugia, Italy, [2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche; Perugia, Italy, [3]Dept. of Science for Nature and Environmental Resources, University of Sassari, Italy, [4]Institute of Biomedical Technologies, National Research Council (ITB-CNR), Milan, Italy, [5]Dept. Biology and Biotechnology, University of Pavia, Italy, [6]Regione Umbria, Osservatorio Faunistico Regionale, Italy

The wild boar is an important game species and the ancestor of domestic pigs. In Italy, recent introductions of exotic individuals and hybridizations have resulted in an uncontrolled population growth, which in turn has threatened the survival of native stocks., Proper management programs are highly improved by genetic characterization. We present here the first molecular study of the wild boar in Umbria. A total of 251 samples, collected in different hunting areas, were screened by analyzing both nuclear microsatellites and mitochondrial DNA (mtDNA). Eighteen nuclear loci were analyzed on 163 samples. Data analyses show three distinct groups clearly separated from the domestic pig. These three groups are geographically separated and divide the Umbria region in three different areas. Boar mtDNA haplotypes (obtained by sequencing 708 bps of the control region) cluster into haplogroups D1, D2 and D4 with the latter being typical of Italy. A fine phylogenetic characterization of entire mitogenomes reveals an ancient divergence of D4 from the

other branches and, provides new clues about the ancestral origin of the Italian wild boar.

P12.6
Bayesian inference of *Pan* evolutionary history inferred from complete mitochondrial genomes

S. Tucci[1], I. Lobon[2], M. de Manuel[2], S. Ghirotto[1], B. Lorente-Galdos[2], J. Hernandez[2], J. Prado-Martinez[2], C. Tyler Smith[3], Y. Xue[3], C. Hvilsom[4], D. Comas[2], G. Barbujani[1], T. Marques-Bonet[2]
[1]Dipartimento di Scienze della Vita e Biotecnologie, Universita` di Ferrara, via Borsari 46, I-44121 Ferrara, Italy, [2]Institut de Biologia Evolutiva, CSIC-UPF, 08003 Barcelona, Spain, [3]The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1HH, UK., [4]Research and Conservation, Copenhagen Zoo, Roskildevej 38, DK-2000 Frederiksberg, Denmark

Humankind's closest living relatives are the two extant species of chimpanzee, *Pan troglodytes* (the common chimpanzee) and *Pan paniscus* (the bonobo). While the bonobo species is not taxonomically subdivided, in chimpanzees four subspecies are recognized which correspond to the geographic ranges where these groups are found in Africa: the Western, the Nigeria–Cameroonian, the Central and the Eastern chimpanzees. Given the lack of fossil records, the understanding of the demographic history of contemporary chimpanzee populations relies almost exclusively in the analysis of genetic data. We analyzed 198 complete *Pan* mitochondrial sequences (mtDNA) collected from different studies, including 20 newly generated sequences. This represents the largest dataset of chimpanzees mtDNA analyzed to date. We investigated the demographic history of the genus *Pan* at the complete mitochondrial level by Approximate Bayesian Computation (ABC). This approach has proved to be particularly powerful to quantitatively compare alternative models and estimate models parameters. Our analysis offer new insights into the forces that shaped the genetic diversity of our closest living relatives.

P12.7
Genetic structure of *Cistus albidus*, a Mediterranean shrub characterised by habitat fragmentation

B Bertolasi[1], L Zago[1], L Loi[1], F Gorian[1], M Zilio[2], C Antognazza[2], I Vanetti[2], G Binelli[2]
[1]Centro Nazionale per lo Studio della Biodiversità Forestale, Peri, Italia, [2]Dept Theoretical & Applied Sciences, Univ. of Insubria, Varese, Italy

The *Cistaceae* are a small family that includes species representative of the earlier successional stages in Mediterranean shrub land ecosystems and dominant in habitats burned by recurrent fires. Most *Cistus* species are widespread with a few narrow endemics patterns resulting from colonization processes after species formation and post-glaciation population survival. *Cistus albidus* L. is a dominant species in degraded habitats and present in scattered populations throughout the western Mediterranean range. In this work we studied by means of SSRs the neutral genetic variability of sixteen populations of *C. albidus*, covering the entire range of the species, with emphasis on Crero, an isolated, possibly relict population from Lake Garda. The Crero population thrives in an unusual environment for a species characteristics of the arid and semi-arid Mediterranean coastal ecosystems. Partial results obtained previously show high genetic variation for only few of the populations, with very high levels of differentiation as indicated by an overall F_{ST} = 0.402. PCA analysis indicates good differentiation between Iberian and Italian populations.

O12.1
Evolutionary history of common bean (*Phaseolus vulgaris L.)*

E. Bitocchi[1], E. Bellucci[1], L. Nanni[1], D. Rau[2], M. Rodriguez[2], G. Attene[2], R. Papa[1]
[1]Dept Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy, [2]Dept Agriculture, University of Sassari, Sassari, Italy

Several genomic tools from molecular markers to Next Generation Sequencing (NGS) data were used to deeply investigate the evolution of common bean. Our findings revealed a Mesoamerican origin of the species with subsequent diverse migrations into South America. Common bean domestication occurred independently in Mesoamerica and Andes. A greater reduction in genetic diversity in Mesoamerica as to the Andes was found, indicating that a bottleneck before domestication occurred in the Andes, which strongly impoverished this wild germplasm, leading to the minor effect of the subsequent domestication bottleneck. Analysis of ~190,000 SNPs from 27,243 contigs in expressed genomic regions showed that not only domestication strongly reduced genetic diversity of domesticated as to wild forms but also had profound effects on gene expression patterns, with contigs of the domesticated frequently showing different levels of expression and patterns of coexpression as to the wild forms. A high percentage of genes (~9%), as to diverse studies in other crops, were found as under selection during domestication. Deep changes at genome level were detected after introduction and spread out of Americas.

O12.2
The horse mitochondrial haplogroup variation in ten Italian local breeds

I. Cardinali[1], H. Lancioni[1], S. Capomaccio[2], K. Cappelli[2], A. Giontella[2], U.A. Perego[1], M.R. Capodiferro[1], E. Rizzi[3], A. Olivieri[4], M. Silvestrelli[2], A. Achilli[1]
[1]Dept. Chemistry, Biology and Biotechnology, University of Perugia, Italy, [2]Dept. Veterinary Medicine, University of Perugia, Italy, [3]Institute of Biomedical Technologies, National Research Council (ITB-CNR), Milan, Italy, [4]Dept. Biology and Biotechnology, University of Pavia, Italy

For more than 5,000 years domestic horse (*Equus caballus*) breeds have been shaped for many different purposes and were involved in human migration events. Also the current Italian horses (regularly recorded in studbooks) are the result of both selection and ancient movements. In this context, genealogical reconstructions and morphological analyses are fundamental to trace these modern breeds back to their origins, but only molecular analyses are suitable for ancient genetic histories reconstruction. Recent studies on horse mitogenomes revealed a complex phylogeny made out of at least 18 different haplogroups (one identified only in the Przewalski horse). Such information was here exploited to define the mitochondrial DNA (mtDNA) variation of ten local Italian breeds. After an accurate genealogical analysis of the maternal lines, DNA samples were collected from 410 unrelated animals. Their mtDNA haplotypes were assigned to all known domestic haplogroups, differentially distributed among the various Italian breeds. Such a variability (eventually brought to whole mtDNA analyses) will shed light on the origin of Italian local breeds and contribute to their preservation and management.

O12.3
Gorilla centromere DNA: an answer to longstanding questions?

CR. Catacchio[1], R. Ragone[2], G. Chiatante[1], M. Ventura[1]
[1]Department of Biology, University of Bari, Bari, Italy, [2]C.A.R.S.O. Consortium, University of Bari, Valenzano (BA), Italy

In multicellular eukaryotes the centromere/kinetochore interaction is responsible for the pairing and segregation of replicated chromosomes. Centromere DNA is described for its low conservation, repetitive nature, quick evolution and protein-binding competence. Among primates, the major class of centromeric DNA is the pancentromeric α-satellite, made of arrays of 171 bp-monomers, repeated in a head-to-tail pattern making up to 5% of each chromosome. α-satellite sequences can either form tandem heterogeneous monomeric arrays or assemble in higher-order repeats (HORs). Gorilla centromere DNA has been barely characterized, and data are mainly based on hybridizations of human alphoid sequences. We isolated and finely characterized gorilla α-satellite sequences,

revealing structure and distribution similarities with other great apes, but also gorilla-specific features, i.e. the existence of an octameric alphoid suprachromosomal family. Finally, our results displayed hints on a possible role of the centromeric protein-B, paving the way to solve the significance of its essential repetitive nature in association with the centromere function and the α-satellite peculiar evolution.

O12.4
Population genetics of a pelagic notothenioid fish along the Antarctic Peninsula

C. Papetti[1,2], C. Agostini[2], T. Patarnello[3], J. Ashford[4], J. Torres[5], L. Zane[2]
[1]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany, [2]Department of Biology, University of Padova, Padova, Italy, [3]Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (Padova), Italy, [4]Center for Quantitative Fisheries Ecology, Old Dominion University, Norfolk, VA, USA, [5]College of Marine Science, University of South Florida, St. Petersburg, FL, USA

Notothenioid fish dominate Antarctic waters in terms of species and biomass. They bear several morphological adaptations to the cold but lack common features such as a swim bladder, resulting in most species being benthic or demersal.*Pleuragramma antarctica* is the only notothenioid with a complete pelagic life cycle and plays a major trophic role in Antarctic waters. In this study, we assessed nine population samples collected along the Antarctic Peninsula (AP) shelf, by genotyping 16 EST-linked microsatellites. We detected genetic homogeneity locally and differentiation among different geographic areas. Nevertheless, the inherent complexity due to genetic differences between length classes suggested that, in *P. antarctica*, geographic differentiation coexists with chaotic genetic patchiness, with divergence not accumulating, but renewed each generation. A complex dispersal pattern is more likely to interact with high recruitment variability and genetic drift that, if combined with areal fragmentation due to temperature increase in the AP and not counterbalanced by gene flow, may lead to reduced resilience of this species.

O12.5
Symbiosis evolution: retracing the tangled path from establishment to obligate association in the *Betaproteobacteria-Euplotes* relationship

V. Boscaro, C. Sigona, G. Petroni, C. Vannini
Dept Biology, University of Pisa, Pisa, Italy

The *Betaproteobacteria-Euplotes* association is an obligatory symbiotic system involving a monophyletic group of *Euplotes* ciliate species and intracellular betaproteobacteria of the species *Polynucleobacter necessarius* or, alternatively, of the candidate species "*Candidatus* Protistobacter heckmanni". The existence of free-living populations of *P. necessarius* which are never associated with *Euplotes*in the natural environment has been proved. Recent data showed that this relationship has been established more than once and that several symbiont-substitution events took place. Comparative genomic analysis highlighted that the *P. necessarius*symbiotic genome is substantially a subset of the free-living one. Its distinctive character is mainly the presence of remnants of horizontally acquired "invasion genes" which probably allowed the establishment of symbiosis. Indeed, trans-infection experiments showed that, if inserted by microinjection, free-living bacteria are able to replace symbiotic ones inside the host. Therefore this symbiotic system represents a perfect model for retracing multiple events of symbiosis establishment and evolution, unravelling the underlying leading mechanisms.

13 - Neurobiology

P13.1
Leigh Syndrome in *Drosophila melanogaster*: morphological and biochemical characterization of *Surf1* post-transcriptional silencing

C. Da-Rè[1], S. Von Stockum[2], A. Biscontin[1], C. Millino[3], P. Cisotto[1], M. A. Zordan[1], M. Zeviani[4], P. Bernardi[2], C. De Pittà[1], R. Costa[1]
[1]Dept of Biology, Univ. of Padova, Padova, Italy, [2]Dept of Biomedical Sciences, Univ. of Padova, Padova, Italy, [3]CRIBI Biotechnology Centre, Univ. of Padova, Padova, Italy, [4]MRC Mitochondrial Biology Unit, Univ. of Cambridge, Cambridge, UK

Leigh Syndrome (LS) is the most common early-onset, progressive mitochondrial encephalopathy usually leading to early death. The single most prevalent cause of LS is occurrence of mutations in the *hSurf1* gene, and LS^{Surf1} patients show a ubiquitous and specific decrease in the activity of mitochondrial respiratory chain complex IV (cytochrome *c* oxidase, COX). *hSurf1* encodes an inner membrane mitochondrial protein involved in COX assembly. We established a *D. melanogaster* model of LS based on the post-transcriptional silencing of *CG9943*, the Drosophila homolog of *hSurf1*. Knock down of *Surf1* was induced (i) ubiquitously, which led to larval lethality; (ii) in the mesodermal derivatives, which led to pupal lethality; (iii) in the central nervous system, which allowed survival; and (iv) at specific developmental stages. A biochemical characterization was carried out in knock down individuals, which unexpectedly revealed defects in all complexes of the mitochondrial respiratory chain (MRC) and in the F-ATP synthase (complex V) in larvae, and a COX-specific impairment in adults. Silencing of *Surf1* expression in *Drosophila* $S2R^{+}$ cells led to loss of COX activity associated with decreased oxygen consumption. We conclude that *Surf1* is essential for COX activity and mitochondrial function in *D. melanogaster*, and provide a new tool that may help clarify the pathogenic mechanisms of LS.

P13.2
The role of Huntingtin in EGFR pathway of non-neuronal cells: evidences in a *Drosophila Melanogaster* model of Hungtinton Disease

M. Finicelli[1], A. Digilio[1], D. Cavaliere[1], MA. Melone[1,2], G. Peluso[1]
[1]Institute of Bioscence and BioResouces (IBBR), CNR, Naples, Italy, [2]Department of Clinical and Experimental Medicine, Second University of Naples, Naples, Italy

Huntington's disease(HD) is a progressive neurodegenerative disorder caused by an abnormal expansion of a polyglutamine(polyQ) tract at the amino terminus of a large cytoplasmatic protein huntingtin(Htt). Since its identification, the normal function of Htt has been subject to extensive investigation. Protein interaction analyses implicate htt in diverse processes, including intracellular trafficking, vesicle transport, postsynaptic signaling, transcriptional regulation, and apoptosis. Recently, it has been reported that polyQ-htt controls the EGFR degradation and recycling in non-neuronal cells from homozygotic HD patients. In order to further evaluate this polyQ-htt involvement in the EGFR pathway in a genetic model system, we decided to use an established *D.melanogaster* model of polyQ toxicity($128Qhtt^{FL}$). In our approach, the entire 3144 aa human HTT protein, with 128 CAG-repeats, was directed to a specific portion of the wing during its development and the EGFR pathway was analyzed. Alteration in the EGFR signaling leads to modifications in vein and extra-vein pattern. Our data will provide further insights about the role of Htt in EGFR signaling pathway in non-neuronal cells.

P13.3
Neuroprotective effects of two creatine salts in brain hippocampal slices

P. Garbati[1], E. Adriano[1], A. Salis[2,4],G. Damonte[3,4], E. Millo[3,4], M. Balestrino[1]
[1]Department of Neuroscience, Ophthalmology, Genetics, Rehabilitation and Maternal-Infantile Sciences, University of Genova, Largo Paolo Daneo 3, 16132 Genova, Italy, [2]Department of Hearth Environmental and Life Science (DISTAV) University of Genova, Corso Europa 26, 16132 Genova, Italy, [3]Department of Experimental Medicine, Section of Biochemistry, University of Genova [4]Center of Excellence for Biomedical Research, University of Genova, Viale Benedetto XV 5, Genova, Italy

Creatine crosses the biological barriers only using the creatine transporter (CrT). In the Creatine Transporter Deficiency, severe and incurable genetic disease, the CrT is defective and the brain is lacking of creatine. Creatine-derived molecules that could cross BBB biological barriers independently of the CrT might be useful to cure this condition. We investigated two creatine salts, creatine ascorbate and creatine gluconate, that could carry creatine across biological barriers by using another transporter. RESULTS: in the presence of GPA 1mM (CrT blocker) both creatine salts delayed the disappearance of the population spike, this suggests that these compounds cross the cellular membranes using a transporter different from the CrT. By contrast, creatine monohydrate (that needs a functioning CrT) was totally inefficient after CrT impairment. Therefore, we found that under normal conditions the two molecules had a neuroprotective effect comparable to that of creatine. When CrT was blocked, only thr two salts slowed down the disappearance of the population spike.

P13.4
period and *timeless* mRNA splicing profiles under natural conditions in *Drosophila melanogaster*

S. Montelli[1,2], G. M. Mazzotta[1], S. Vanin[1,3], L. Caccin[1], S. Corrà[1], C. De Pittà[1], E. W. Green[4], C. P. Kyriacou[4], R. Costa[1]
[1]Department of Biology, University of Padova, Padova, Italy, [2]Present address: Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, PD, Italy, [3]Present address: School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield, UK, [4]Department of Genetics, University of Leicester, Leicester, UK

During the last years several studies systematically compared *Drosophila* circadian rhythms in laboratory and natural environment. At the molecular level, a strong seasonal influence was observed on PER and TIM natural profiles in the clock cells, opening new questions about the oscillation of *per* and *tim* mRNAs and their post-transcriptional regulation. In the laboratory, thermal sensitive splicing events have been shown to originate two alternative transcripts for both genes. In particular a thermo-sensitive alternative splicing of an 89bp intron in the 3' UTR of *per* transcript generates two isoforms, resulting in an earlier onset of the evening locomotor activity at cold temperature. As for *tim*, at cold temperature the last 858bp intron is retained and the corresponding transcript originates a TIM protein ($TIM^{UNSPLICED}$) 33 amino acids shorter than the full length, due to a premature STOP codon. We have studied *per* and *tim* mRNAs profiles in natural conditions throughout the seasons, focusing on the different isoforms regulated by the thermo-sensitive splicing and we speculate about the possible role of the $TIM^{UNSPLICED}$ protein.

P13.5
Morning serum melatonin in patients with mood disorders and insomnia: influence of age, body mass index, smoke and photoperiod

L. Palego[1], I. Masala[1], L. Betti[2], L. Palagini[1], F. Luchini[1], B. Pacciardi[1], A. Lucacchini[2], M. Mauri , G. Giannaccini[2]
[1]Department of Clinical and Experimental Medicine, University of Pisa, Italy, [2]Department of Pharmacy, University of Pisa, Pisa, Italy

Aim of this study was to evaluate morning (9.00) serum melatonin (MLT), the pineal gland sleep-wake hormone, in a total of 18 patients (12W/6M) with mood disorders and insomnia relatively to subjects'age,

body mass index (BMI, kg/m^2), smoke and photoperiod (winter/spring). Melatonin (pg/ml) was determined before (t_0) and after treatment (t_1) with the pro-hypnotic drug Trazodone (TRZ) using HP-LC with electrochemical-fluorimetric detection. Results showed a non statistically significant trend toward reduced $t_1$serum MLT, lower at wintertime, while therapy being significantly successful. No age effect was reported, while smokers had significantly reduced MLT levels at t_1. Significant differences/negative correlations of MLT as a BMI function were obtained at t_0 and t_1. Conclusively, morning serum MLT did not appreciably vary with insomnia outcome in these mood-disorder patients, differently from our previous data on night urinary MLT metabolite. The reported link between lower morning MLT and higher BMIs suggests that body-weight gain and MLT bio-clock dysfunctions are interlaced in affective disturbances, perhaps as trait correlates.

P13.6
Unstable dendritic spines in a mouse model of CDKL5 disorder

G. Della Sala[1], E. Putignano[2], G. Chelini[1], E. Calcagno[3], G. M. Ratto[4], E. Amendola[5], C. Gross[5], M. Giustetto[3], T. Pizzorusso[1,2]

[1]Department of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA University of Florence, Area San Salvi – Pad. 26, 50135 Florence, Italy, [2]Institute of Neuroscience, National Research Council (CNR), Via Moruzzi 1, 56124 Pisa, Italy, [3]University of Turin, Department of Neuroscience and National Institute of Neuroscience, Corso M. D'Azeglio 52, 10126 Turin, Italy, [4]NEST, Scuola Normale Superiore, Nanoscience Institute-CNR, Pisa, Italy, [5]Mouse Biology Unit, European Molecular Biology Laboratory (EMBL), via Ramarini 32, 00015 Monterotondo, Italy

CDKL5 is mutated in many severe neurodevelopmental disorders, including some forms of atypical Rett syndrome. An in vivo analysis of the role of CDKL5 in dendritic spine dynamics and synaptic molecular organization is still lacking. we performed in vivo two-photon imaging of pyramidal neurons in the somatosensory cortex of male CDKL5 null mice. We found that adult mutant mice (imaged from P50 until P80) show a significant reduction in spine density that remained substantially stable during the observation period. Short-term spine turnover was unaffected, however stable spines persisting for one month were significantly reduced. Spine deficits were accompanied by synaptic deficits consisting in a reduction of synaptic PSD95 and impaired LTP. In young CDKL5 null mice spine density and levels of synaptic PSD95 already begin to be reduced at this age. Moreover, repeated in vivo imaging showed a dramatic increase in short-term spine elimination but normal spine formation. To explore a possible therapeutical approach to reverse synaptic deficits, we administered IGF1 to juvenile mutant mice and we found that both spine density and spine loss rate were rescued to control levels.

P13.7
Delivery of a neuroprotective cell-penetrating Hsp70

L. Pollegioni[1,2], P. Cappelletti[1,2], M. Tunesi[3,4], S. Rodilossi[5], D. Albani[5], C. Giordano[2,3,4]

[1]Dept. Biotechnologies and Life Sciences, Università degli Studi dell'Insubria, Varese, Italy, [2]'The Protein Factory', Politecnico di Milano, ICRM-CNR Milano and Università degli Studi dell'Insubria, Varese, Italy, [3]Dept. Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Italy, [4]Unità di Ricerca Consorzio INSTM, Politecnico di Milano, Milan, Italy, [5]Dept. Neurosciences, "Mario Negri" Institute for Pharmacological Research, Milan, Italy

Parkinson's disease is a severe CNS disorder characterized by the progressive loss of dopaminergic neurons. Seeking for innovative therapies, we focused on the setup of a delivery system of the neuroprotective protein Hsp70. Wild-type and cell-penetrating Tat-fused human Hsp70 were expressed in *E. coli* as soluble proteins with a purity >95% and a yield of 95 and 12 mg/L, respectively. The quality of the recombinant protein preparations was verified by CD spectroscopy and activity assay. Then we designed suitable hydrogel-based systems as protein delivery device (to avoid limitations related to blood-brain barrier transport). Protein release experiments showed that TAT-Hsp70 is more slowly released than Hsp70. The SH-SY5Y cell line was used as *in vitro* model of neurotoxicity: the cells were treated with the dopaminergic-selective toxin 6-OHDA and the neuroprotective activity of the released Hsp70 and TAT-Hsp70 was assessed. The MTS assay set at 250 nM Hsp70 or TAT-Hsp70 concentration, at which the proteins were not detrimental to SH-SY5Y cells: a protection against 6-OHDA toxicity was observed.,*We thank Fondazione Cariplo (Grant no: 2011-0335) for financial support.*

P13.8
Magnetic nanoparticles can direct axonal growth

C. Riggio[1], M.P. Calatayud[2], M. Giannaccini[3], B. Sanz[2], T.E. Torres[2], R. Fernández-Pacheco[2], M.R. Ibarra[2], J. del Río[4], L. Dente[3], A. Cuschieri[1,5], G.F. Goya[2], V. Raffa[1,3,5]

[1]Institute of Life Science, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127, Pisa, Italy, [2]Instituto de Nanociencia de Aragón, Universidad de Zaragoza, Mariano Esquillor 50018, Zaragoza, Spain, [3]Department of Biology, Università di Pisa, S.S. 12 Abetone e Brennero 4, 56127, Pisa, Italy, [4]Molecular and Cellular Neurobiotechnology, Institute for Bioengineering of Catalonia (IBEC), Barcelona Science Park, Barcelona 08028, Spain, [5]IMSaT, University of Dundee, Dundee Medipark, Dundee DD2 1FD, UK

The importance of mechanical factors for the nervous system has been appreciated only recently. It has been demonstrated that cellular tension is a crucial factor in neuronal development and axonal growth. Recently we demonstrate in neuron like cells that magnetic nanoparticles (MNPs) can be a powerful nanotool for application of very low tensions (range of pN) which could be used to systematically investigate the role of force on neurite growth and orientation at long time scales (days). Current work is focused on translation of this finding to a mouse organotypic model of nerve regeneration. The model consists of an organotypic slice of spinal cord co-cultured with a sciatic nerve graft. This regeneration model showed that axons from the motor neurons of the ventral root of spinal cord re -innervated the sciatic nerve by grossing the gap (site of lesion) between the two explants. Specifically the interactions of MNPs with the regenerating axons of motor neurons are under investigation.

P13.9
Engineering membrane models capable to target β-amyloid and to modulate its aggregation features: implications for therapy of Alzheimer's disease

F. Re[1], C. Balducci[2], S. Minniti[1], S. Mancini[1], A. Cagnotto[3], M. Salmona[3], G. Forloni[2], M. Masserini[1]

[1]Department of Health Sciences, University of Milano-Bicocca, Monza, Italy, [2]Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy, [3]Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy

The over-production and aggregation of the neurotoxic β-amyloid peptide (Aβ) are critical processes in Alzheimer's disease (AD). We developed membrane models (liposomes, LIP) composed of sphingomyelin/cholesterol/phosphatidic acid (PA) and surface decorated with a modified peptide from the ApoE receptor-binding domain (mApoE). These LIP are able to bind with high affinity Aβ (Kd=0.6μM by SPR), to inhibit Aβ aggregation (70%inhibition after 72h) and trigger its disaggregation (60%decrease after 120h incubation), thanks to a synergic action of PA/mApoE, as suggested by Molecular Dynamics Simulation studies. Radiochemical experiments, suggest that LIP are able to cross the BBB by receptor-mediated transcytosis, either *in vitro* (permeability=2.5x10-5cm/min, 5-fold higher respect to control LIP) or *in vivo* in healthy mice (0.3% of the injected dose, 3-fold higher respect to control LIP). Repeated I.P. administration of LIP to transgenic mice ameliorated impaired memory, as assessed by NOR test and reduced brain Aβ levels (-29.8%, P<0.05), in particular oligomers (-57.5%, P<0.01). Overall, we can hypothesized that LIP reaching the brain could act as "catalysers" of

Aβ disaggregation, while the large amount of LIP present in the blood could remove circulating Aβ, enhancing its passage brain-to-blood and its peripheral clearance (the so called 'sink effect') for a potential AD treatment

P13.10
Enhanced expression of amyloid precursor protein in platelets from patients with Alzheimer's Disease and Frontotemporal Lobar Degeneration

D. Sartini[1], E. Salvolini[2], S. Morganti[1], V. Pozzi[1], S. Luzzi[3], R. Fiorini[4], L. Provinciali[3], R. Di Primio[2], L. Mazzanti[1], M. Emanuelli[1], A. Vignini[1]
[1]Dipartimento di Scienze Cliniche Specialistiche e Odontostomatologiche, Università Politecnica delle Marche, Ancona, Italy, [2]Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche, Ancona, Italy, [3]Dipartimento di Medicina Sperimentale e Clinica, Università Politecnica delle Marche, Ancona, Italy, [4]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy

Alzheimer's Disease (AD) and Frontotemporal Lobar Degeneration (FTLD) represent two of the most frequent causes of degenerative dementia. AD is characterized by the presence of beta-amyloid (Aβ) plaques, distributed throughout the cerebral cortex. Platelets are an important peripheral source of amyloid precursor protein (APP). They possess the enzymatic machinery to produce Aβ and thus offer an ex vivo model to study APP processing and changes associated with AD. The aim of the present study was to evaluate mRNA expression level of total APP (TOT) and APP containing a Kunitz-type serine protease inhibitor domain (KPI) in platelets from patients with AD and from patients with FTLD, as well as from healthy subjects. Moreover, the correlation between platelet APP mRNA expression levels and patient cognitive impairment was evaluated. Differential gene expression measurements revealed a significant upregulation of APP TOT and APP KPI in both AD and FTLD patients, compared to the controls. Interestingly, in AD patients APP mRNA expression levels positively correlated with cognitive impairment, whereas in FTLD patients this correlation did not reach the statistical significance.

P13.11
CLIC1 is relevant for exosome-dependent growth of glioma cells

M. Setti[1], C. Richichi[1], D. Osti[1], M. Del Bene[1], G. Beznusenku[2], A. Mironov[2], A. Lorico[3], G. Pelicci[1,4]
[1]Department of Experimental Oncology, European Institute of Oncology, Milan, Italy, [2]FIRC Institute of Molecular Oncology, Milan, Italy, [3]Cancer Research Center, Roseman University of Health Sciences, Las Vegas, NV 89135, USA, [4]Department of Translational Medicine, Università del Piemonte Orientale, Novara, Italy

Little progresses have been made in the treatment of glioblastoma (GBM), the most aggressive and lethal among brain tumors. Recently we have demonstrated that Chloride Intracellular Channel-1 (CLIC1) is overexpressed in GBM compared to normal tissues with highest expression in those patients with poor prognosis. CLIC1-silencing in cancer stem cells (CSCs) isolated from human GBM patients negatively influences proliferative capacity and self-renewal properties *in vitro*and impairs the *in vivo* tumorigenic potential. Exosomes, 40-120 nm microvesicles (MVs) arising from the invagination of the limiting membrane of late endosomes, have been shown to sustain and support tumor growth in a variety of model systems, including GBM. We isolated MVs from both GBM cell lines and primary cultures from GBM patients and CLIC1 was detectable in purified MVs through western blot and electro-microscopy. GBM cells treated with autologous MVs showed an increased proliferative capacity both *in vitro* and *in vivo*; we isolated MVs from CLIC1-interfered or CLIC1-overexpressing GBM cells and proved that the MVs-mediated positive response was strongly CLIC1-dependent. CLIC1 modulation apparently did not alter MVs biogenesis or global structure. Our findings might help to unravel the tumorigenic role of CLIC1 in GBM and the biological function of exosomes whose good therapeutic potential and clinical exploitability is of absolute scientific value.

P13.12
A P2X7 antagonist improves muscle strength and motor distal amplitude in CMT1A rats: preliminary results from an in vivo trial

G. Sociali[1], T. Prukop[2], I. Cervellini[2], L. Nobbio[3], S. Bruzzone[1], A. De Flora[1], A. Schenone[3], M. W. Sereda[2]
[1]Department of Experimental Medicine, Section of Biochemistry, and CEBR, University of Genova, Italy, [2]Max Planck Institute of Experimental Medicine, Department of Neurogenetics, Göttingen, Germany, [3]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Mother and Child Sciences and CEBR, University of Genova, Italy

Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy, and a duplication of the *Pmp22* gene causes the most frequent subform CMT1A. An abnormally high intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) occurs in Schwann cells (SC) from CMT1A rats and is caused by overexpression of the P2X7 purinoceptor. Normalization of Ca^{2+} levels through down-regulation and pharmacological inhibition of P2X7 appears to restore the normal phenotype of CMT1A SC in vitro. Here, we investigated whether treatment with a P2X7 inhibitor (A438079) improves CMT1A disease in vivo. We applied placebo-controlled A438079 therapy (3 mg/Kg) to CMT1A rats and performed the grip strength analysis and electrophysiology. After 6 weeks of treatment, A438079 significantly increased muscle strength of CMT1A rats. Moreover, a preliminary analysis indicates that compound action potentials (CMAP) were increased in treated CMT1A rats indicating that axonal loss may be ameliorated. Antagonizing P2X7 could represent a promising strategy for CMT1A. Morphological analysis on nerves from treated and control animals are currently being performed, together with RT-PCR analysis of SC differentiation markers.

O13.1
Estrogen regulates neuroglobin transcription in neuronal and non-neuronal cancer cells

S. Reineri[1,2], L. Vanzan[2], G. Ferrero[1,3], F. Cordero[1,3], M. Fiocchetti[4], M. Marino[4], P. Ascenzi[4,5], M. De Bortoli[1,2], S. Cutrupi[1,2]
[1]Center for Molecular Systems Biology, University of Turin, Turin, Italy, [2]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy, [3]Department of Computer Science, University of Turin, Turin, Italy, [4]Department of Science, University Roma Tre, Roma, Italy, [5]Interdepartmental Laboratory of Electron Microscopy, University Roma Tre, Roma, Italy

Neuroglobin (NGB) is a brain globin with neuroprotective effects, acting as O_2 sensor and antiapoptotic pathway transducer. NGB expression is induced by E2 suggesting a new mechanism of E2 in neuroprotection. In MCF7 and HepG2 non-neuronal cancer cells, E2 induced NGB levels through ERα that is inhibited by the transcription inhibitor actinomycin D. Using bioinformatic analysis based on ENCODE database, we observed a DNase-hypersensitive site signal in the first NGB intron, overlapping H3K27ac and p300 signals, indicating that NGB first intron contains an active enhancer. Putative ERE were also identified at both enhancer and promoter. Interestingly,coexpression of NGB with ERα was found in the hypothalamus in the Allen Human Brain Atlas. Using the human SK-N-BE neuroblastoma cell line and ChIP analysis, we observed that E2 treatment induced ERα binding to both NGB promoter and enhancer and resulted in increased recruitment of active P-PolII at the same sites. These two regulatory sequences were also marked by H3K4me3 and H3K27me3 histone modifications suggesting that they were modified upon E2 stimulation. Estrogen may act directly on the NGB gene through estrogen receptors.

O13.2
miR-135a and the regulation of neuronal plasticity under stress conditions

C. Mannironi[1], A. Biundo[2], F. De Vito[3], S. Caioli[3], C. Zona[3,4], T. Ciotti[5],

I. Bozzoni[2], A. Mele[2,5,6], C. Presutti[2]
[1]Istituto di Biologia, Medicina Molecolare e Nanotecnologie, CNR, Roma, [2]Dipartimento di Biologia e Biotecnologie "C.Darwin" Sapienza Universita' di Roma, Roma, [3]IRCCS Fondazione S. Lucia, Roma, [4]Dipartimento di Medicina dei Sistemi, Università di Roma Tor Vergata, Roma, [5]Istituto di Biologia Cellulare e Neurobiologia, CNR, Roma, [6]Centro di Ricerca in Neurobiologia, D. Bovet, " Sapienza Universita' di Roma, Roma

In the brain, stress-related information from all sensory systems is conveyed to limbic brain structures, in particular the amygdala is considered a key node for stress response integration. Adaptation to stress involves highly coordinated changes in gene expression, involving different layers of post-transcriptional regulation. We have recently shown (Mannironi et al, 2013) that microRNA miR-135a, is down-regulated by acute restraint stress. Moreover, we found that miR-135a modulates mineralcorticoid receptor expression, one of the key effectors of the corticosteroid cascade, and a crucial component of the stress signaling response in amygdala. In these new experiments we further characterized miR-135a demonstrating its role in the post-transcriptional regulation of two major components of the synaptic fusion complex, Complexin 1 and 2 (Cplx1, 2). First we showed that over-expression of miR-135a strongly reduces the expression of Cplx1 and Cplx2 3'UTR luciferase reporters, as well as CPLX1 and 2 endogenous mRNA and protein levels. We also found that miR-135a knocked-down in hippocampal neurons enhanced Cplx1 and 2 expression and strongly affected the excitatory neurotransmission.

O13.3
Experience-dependent DNA methylation regulates plasticity in the developing visual cortex

D. Napoli[1,5], P. Tognini[1], J. Tola[1], D. Silingardi[1], F. Della Ragione[3,4], M. D'Esposito[3,4], T. Pizzorusso[1,2]
[1]InstituteofNeuroscience CNR, via Moruzzi, 1 56100 PISA, ITALY, [2]Department of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA, University of Florence, Via di San Salvi 12, Padiglione 26, 50135 Firenze, Italy, [3]IGBA.B.T.CNR via P. Castellino, 111 Napoli, Italy, [4]IRCCS NeuromedPozzilli (Is) Italy, [5]ScuolaNormaleSuperioredi Pisa, Pisa, Italy

DNA methylation is an epigenetic repressor mark for transcription dynamically regulated in postmitotic neurons. We analyzedvisual experience regulation of DNA methylationand its involvement in ocular dominance plasticity (ODP) of the developing visual cortex. Monocular deprivation increased DNA methyltransferases (DNMTs) expression and exerted opposite effects on DNA methylation and hydroxylmethylation in specific promoters of plasticity genes. Inhibition of DNMTs blocked molecular and functional effects of monocular deprivation partially reversing monocular deprivation transcriptional program.

O13.4
Mutations in the mitochondrial citrate carrier SLC25A1 are associated with impaired neuromuscular transmission

V. Porcelli[1], A. Chaouch[2], P. Scarcia[1], D. Cox[2], A. De Grassi[1], S.H. Laval[2], C.L. Pierri[1],H. Griffin[2], J.S. Müller[2], T. Evangelista[2], V. Straub[3], R. Horvath[3], S. Edvardson[4], A. Sadaa[4], O. Elpeleg[4], H. Lochmuller[2], L. Palmieri[1,5]
[1]Department of Biosciences, Biotechnology and Biopharmaceutics, Laboratory of Biochemistry and Molecular Biology, University of Bari Aldo Moro, Bari, Italy, [2]Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK, [3]Institute of Human Genetic, Newcastle University, Newcastle upon Tyne, UK, [4]Monique and Jacques Roboh Department of Genetic Research, Hadassah, Hebrew University Medical Center, Jerusalem, Israel, [5]CNR Institute of Biomembranes and Bioenergetics, Bari, Italy

The mitochondrial citrate carrier, encoded by the *SLC25A1* gene, catalyzes the export of citrate or isocitrate from mitochondria in exchange for cytosolic malate.*SLC25A1*has recently been linked to a severe, often lethal clinical phenotype. By homozygosity mapping and whole exome sequencing, we identified a novel homozygous missense mutation in the *SLC25A1*gene in a sib pair with a milder phenotype presenting primarily as a neuromuscular junction (NMJ) defect. *In vitro* assays showed abnormal carrier function. Yet the newly indentified mutation caused a milder activity impairment than previously reported mutations. Using knockdown of SLC25A1 expression in zebrafish, we were able to mirror the human disease in terms of variable brain, eye and cardiac involvement. Importantly, we show clear abnormalities in the NMJ, regardless of the severity of the phenotype. Of note, a previously reported patient with different compound heterozygous missense mutations of *SLC25A1*has since been shown to suffer from a NMJ defect. Based on the axonal outgrowth defects seen in SLC25A1 knockdown zebrafish, we hypothesize that the NMJ impairment may be related to pre-synaptic nerve terminal abnormalities.

14 - Cell Communication, Cell Adhesion and Membrane Trafficking

P14.1
Electrical activity dependent cx43 acetylation: a new mechanism for regulating cardiomyocyte communication

V. Azzimato[1], V. Meraviglia[2], C. Colussi[3], C. Florio[2], A. Panariti[4], K. Qanud[5], C. Gaetano[6], P. Pramstaller[2], M.C. Capogrossi[7], G. Pompilio[8], F. Recchia[9], I. Rivolta[4], A. Rossini[2].
[1]Univ di Milano, Milano, Italy, [2]European Acad of Bozen, Bolzano, [3]Univ Cattolica del Sacro Cuore, Roma, [4]Univ of Milano Bicocca, Monza, [5]New York Medical Coll, Valhalla, NY, [6]Goethe Univ, Frankfurt am Main, Germany, [7]Istituto Dermopatico dell'Immacolata, Roma, Italy, [8]Temple Univ Sch of Med, Philadelphia, PA, [9]Ctr Cardiologico Monzino, Milano, Italy

Communication among cardiomyocytes depends upon Gap Junction (GJ) proteins.Previous studies demonstrated that electrical stimulation can induce GJ remodeling and may influence Lysine acetylase (KAT) and deacetylases (KDAC) activities.Aim of this work was to establish whether electrical stimulation modulates cell-cell communication by acetylation dependent mechanisms.24 hours electric stimulation caused connexin 43 (Cx43) reduction both in neonatal rat cardiomyocytes and HL-1cells, while not in human fibroblasts.Confocal microscopy revealed that electrical stimulation induced Cx43 accumulation in HL-1 cytoplasm.Further, chronic pacing significantly down-regulated KDAC activity, whereas KAT activity was not modified, resulting in a general increase of cell protein acetylation and confirmed by western blot analysis.The pacing-dependent acetylation of Cx43 was proven by immunoprecipitation assay.The treatment of paced cells with the KAT inhibitor Anacardic Acid was able to rescue Cx43 level.Intriguingly, preliminary results also indicate lateralization and increased acetylation of Cx43 in the left ventricles of dogs with pacing-induced dilated cardiomyopathy. In conclusion, electrical stimulation of cardiac cells promotes Cx43 acetylation, leading to Cx43 down-modulation and intracellular relocalization.These findings suggest that electrical activity-dependent increase in acetylation may represent a novel mechanism for the regulation of cardiomyocyte communication.

P14.2
Quantification of tissue T3, T4 and their metabolites in rat and human tissues by a novel HPLC-MS/MS method

R. Donzelli[1], A. Saba[1], D. Colligiani[2], M. Nannipieri[2], C. Kusmic[3], C. Dos Remedios[4], W. Simonides[5], G. Iervasi[3,6], R. Zucchi[1]
[1]Department of Pathology, University of Pisa, Pisa, Italy, [2] Department of Medicine, University of Pisa, Pisa, Italy, [3] National Research Council, Istitute of Clinical Physiology, Pisa, Italy, [4] Muscle Research Unit, Bosch Institute, The University of Sydney, Sydney, Australia, [5] Laboratory for Physiology, Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, The Netherlands, [6]G. Monasterio Foundation, Pisa, Italy

We report a novel technique based on tandem mass spectrometry coupled to HPLC, which enables to assay thyroid hormone (T3), thyroxine (T4) and their metabolites (such as 3-iodothyronamine, T1AM) concentrations which are difficult to assay in tissues by conventional immunological methods. The major difference vs. previous HPLC-MS/MS based methods lies in the derivatization step with 3 M HCl in n-butanol that provides butyl esters of T3 and T4. Through our method human or animal tissues are spiked with labeled internal standards, homogenized, extracted and derivatized as described. Our method allowed detection of T3 and T4 in human left ventricle biopsies yielding concentrations of 1.51±0.16 and 5.94±0.63 pmol/g, respectively. In rats treated with different dosages of synthetic T3 or T4, good correlations ($r>0.90$) between plasma and myocardial T3 and T4 concentrations were observed, although in specific subsets different plasma T4 concentration were not associated with different tissue content in T4. We conclude that this method could provide a novel insight into the relationship between plasma and tissue thyroid hormone levels.

P14.3
Plasma membrane sialidase NEU3 controls epithelial-mesenchymal transition in melanoma cells

I. Silvestri[1], F. Testa[1], F. Benigni[2], G. Grazia[3], V. Perotti[3], P. Baldassari[3], R. Mortarini[3], A. Anichini[3], B. Venerando[1], C. Tringali[1]
[1]Dept. of Medical Biotechnology and Translational Medicine, University of Milan, Segrate, Milan, Italy, [2]San Raffaele Scientific Institute, Urologic Research Institute (URI),Via Olgettina 60, Milan, Italy, [3]Human Tumors Immunobiology Unit, Dept. of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Melanoma is a highly aggressive tumour with poor prognosis in the metastatic stage. To metastasize, melanoma cells undergo epithelial mesenchymal transition (EMT). We demonstrated that membrane sialidase NEU3, which is highly over-expressed in melanomas, is strictly involved in EMT control. We showed that melanomas can be grouped into three clusters based on their ganglioside profile, associated with different malignant properties and patients' survival. In cluster 1, the high activity of NEU3 contributes to reduce the levels of N-acetyl GM3 in favour of N-glycolyl GM3. In fact, NEU3 silencing promoted the increase of N-acetyl GM3 and reverted EMT, as demonstrated by the modified expression of EMT markers (E-cadherin N-cadherin, TGF-b receptor, and integrins, including b1, b3, b4 and a5). NEU3 was co-immunoprecipitated with TGF-b receptor and GM3; therefore, it is realistic that alterations of NEU3 levels could modify gangliosides surrounding TGF-b and thus its activation. NEU3 silenced melanoma cells displayed a marked reduction of migration, growth in soft agar and *in vivo*. Based on these results, sialidase NEU3 could be considered a novel target in melanoma therapy.

P14.4
AQP8 facilitates Nox-produced hydrogen peroxide transport in leukaemia cells

F. Vieceli Dalla Sega[1], L. Zambonin[1], D. Fiorentini[1], B. Rizzo[2], C. Prata[1], S. Hrelia[2]
[1]Department of Pharmacy and Biotechnology, University of Bologna, Italy, [2]Department for Life Quality Studies, University of Bologna, Italy

It is known that an increase in the intracellular level of reactive oxygen species (ROS), in particular hydrogen peroxide (H_2O_2), is able to affect signalling pathways regulating proliferation and cancer development. H_2O_2 has been long thought to freely permeate across biological membranes, but recently, new evidence demonstrated that membrane H_2O_2-permeability is limited and that specific aquaporin (AQP) isoforms are able to funnel H_2O_2 across membranes. Data here reported show that AQP inhibition cause a decrease in intracellular H_2O_2 accumulation in leukaemia cells. Furthermore, AQP8 overexpression or silencing by means of siRNA resulted in the modulation of VEGF ability of triggering H_2O_2 intracellular level increase or decrease, respectively. Finally, we show that AQP8-facilitated H_2O_2transport is able to increase cell proliferation through a mechanism dependent on PI3K and p38MAPK. In summary, our findings indicate that AQP8 is able to modulate H_2O_2 transport through the plasma membrane affecting redox signalling linked to leukaemia cell proliferation . Therefore, the development of new drugs targeting specific AQP isoforms might be an interesting novel anti-cancer strategy.

O14.1
Diacylglycerol kinase alpha regulates mitotic spindle orientation during epithelial morphogenesis

V. Bettio[1], E. Rainero[1], S. Faletti[1], D. Capello[1], D.M. Bryant[2], A. Datta[2], K.E. Mostov[2], A. Graziani[1]
[1]Department of Clinical and Experimental Medicine, University of Piemonte Orientale Amedeo Avogadro, Novara, Italy, [2]Department of Anatomy, UCSF, San Francisco, CA USA 94143

Epithelial cells cultured in 3D ECM organize in polarized, hollowed cysts, with an apical domain (AP) facing the lumen and a basolateral domain (BL) in contact with the ECM. Diacylglycerol kinases (DGKs) phosphorylate diacylglycerol (DG) to phosphatidate (PA), regulating DG- and PA-mediated signalling. DGKa-generated PA mediates growth factors-induced cell migration and invasion by recruiting the aPKCs/RhoGDI/Rac1 complex and Rab11-FIP1, regulating Itga5b1 recycling. In MDCK cysts, DGKa activity is required for proper cystogenesis, since DGKa silcenced/inhibited cysts show multiple lumen. DGKa localizes at the BL and Its activity is necessary to i) recruit AnxA2 and Cdc42 to the AP, ii) segregate PIP_3 to BL, iii) orientate the mitotic spindle in parallel to the central lumen. The multiple lumen phenotype is also PLDs-mediated, since inhibiting both DGKa and PLDs the single lumen is restored. Thus DGKa is a key regulator of epithelial morphogenesis by regulating vesicular trafficking and mitotic spindle orientation. The DGKa-mediated molecular mechanisms may involve Itg-recycling and EGFR-dependent astral microtubule anchorage to the cell cortex via a DGKa/PLD-regulated PA balancing.

O14.2
Synthetic agents for protein dimerization, a bridge between chemistry and biology for protein characterization and potential drug development.

E. Da Pozzo, C. Giacomelli, L. Rosalia, E. Nuti, C. Martini, A. Rossello
Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy

Protein homodimers play important roles in physiological and pathological processes. Matrix metalloproteases (MMPs) are used here as a model system to study and control protein homodimerization. Starting from described twin hydroxamate inhibitors, able to induce the MMP dimerization, the effects of varying linker lengths were investigated on both isolated enzymes and cell cultures. The inhibitory enzyme activities of the new molecules were demonstrated on human recombinant MMPs by fluorometric assay. Moreover, the compounds were able to induce MMP9 dimerization in tube as evidenced by western blotting analyses. The new molecules have been used as readout system on two human glioblastoma cancer cell lines expressing MMP-9 and MMP-12 at different levels; inhibitors were able to statistically reduce the MMP mRNA levels, without affecting cell proliferation or survival, but greatly arresting cell invasiveness, mainly in cells expressing high MMP levels. On these basis, these selective synthetic MMP inhibitors could be potentially used to investigate the mechanisms of homo- and/or hetero-dimerization described for MMP enzymes (CritRevBiochemMolBiol 2013, 48, 222-272).

O14.3
PDK1 regulates focal adhesion disassembly through modulation of αvβ3 integrin endocytosis

L. di Blasio[1,2], P.A. Gagliardi[1,2], A. Puliafito[1,2], R. Sessa[1,2,3], G. Seano[1,2,4], F. Bussolino[1,2], L. Primo[1,2]
[1]Candiolo Cancer Inst., Candiolo, Turin, Italy, [2]Deptt of Oncology, Univ.y of Torino, Turin, Italy, [3]Current address: UC Berkeley, 689 Minor Hall, Berkeley, USA, [4]Current address: Edwin Steele Laboratory for Tumor Biology, Department of Radiation Oncology, Harvard Medical School, Massachusetts General Hospital, Boston, USA

Non-amoeboid cell migration is characterised by dynamic competition among multiple protrusions to establish new adhesion sites at the leading edge. However, the mechanisms that regulate the decision to disassemble or to grow nascent adhesions are not fully understood. Here we show that in endothelial cells (EC) 3-phosphoinositide-dependent protein (PDK1) promotes focal adhesions (FA) turnover by controlling endocytosis of integrin αvβ3 in a PI3K-dependent manner. We demonstrate that PDK1 binds integrin αvβ3 and induces its endocytosis. Down-regulation of PDK1 increases the FA size and slows down their disassembly. This process requires both PDK1 kinase activity and PI3K activation but does not involve Akt. Moreover, PDK1 silencing stabilizes FA in membrane protrusions decreasing EC migration on vitronectin. These results indicate that modulation of integrin endocytosis by PDK1 hampers EC adhesion and migration on extracellular matrix, thus unveiling a novel role for this kinase.

O14.4
Dyskerin depletion perturbs cell migration and adhesion in colon cancer cells

N. Di Maio, V. Morra, A. Palumbo, A. Angrisani, M. Furia, M. Turano
Dept of Biology, University of Naples Federico II, Italy

Human dyskerin is an evolutively conserved protein whose deficiency causes the X-linked dyskeratosis congenita disease. Cancer susceptibility represents a main characteristic of X-DC, with head and neck squamous cell carcinomas most frequently observed, followed by skin and rectal cancer. Dyskerin depletion perturbs cell cycle progression and proliferation causing a G2/M arrest, as observed in fibroblasts from dyskeratosis congenita patients and in transformed dyskerin depleted cells. With the aim to understand the apparent contradictory link between decreased cell proliferation and cancer susceptibility observed in dyskeratosis congenita, we generated a stable colon carcinoma cellular model capable of down-regulating dyskerin accumulation following administration of tetracycline or its more stable derivative doxycycline. We found that depletion of dyskerin function inhibits growth and alters cell morphology. Moreover we observed that down-regulation of dyskerin determines alteration of cell-matrix adhesion and increasing of cell migration in silenced cells. In conclusion, our data shed more light into the understanding of dyskerin contribution to tumorigenesis.

O14.5
Abscisic acid transport in human erythrocytes

T. Vigliarolo, L. Guida, A. De Flora, E. Zocchi
Department of Experimental Medicine, Section of Biochemistry, University of Genova, Genova, Italy

Abscisic acid (ABA) is a plant hormone involved in the response to environmental stress. Recently, ABA has been shown to be present and active also in mammals, where it stimulates the functional activity of innate immune cells and insulin release. LANCL2, the ABA receptor in mammalian cells, is located at the intracellular side of the plasma membrane. Here we investigated the mechanism enabling ABA transport across the plasmamembrane of human erythrocytes (RBCs). Both influx and efflux of [H^3]-ABA occur across intact RBCs, as detected by radiometric and chromatographic methods. ABA binds specifically to Band 3 (the erythrocyte anion transporter), as determined by labeling of RBCs membranes with biotinylated ABA. Proteoliposomes reconstituted with human purified Band 3 transport [H^3]-ABA and [S^{35}]-sulphate and ABA transport is inhibited by the specific Band 3 inhibitor DIDS. Once inside RBCs, ABA stimulates ATP release via pannexin through the LANCL2-mediated activation of adenylate cyclase. These results suggest a role for plasma ABA in the vasodilator response activated by ATP released from RBCs in response to environmental stress (hypoxia, low pH, shear stress).

15 - Oncogenes and Tumor suppressors

P15.1
Oncogenic-promoting activity of 14-3-3θ in t(4;11) acute lymphoblastic leukemia

T. Fioretti[1], A. Cevenini[1,2], D. Sarnataro[1,2], F. Manfrevola[1], A. Perna[1], F. Salvatore[1,2], G. Esposito[1,2]
[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II", Napoli, Italy, [2]CEINGE-Biotecnologie Avanzate S.c.a R.L., Napoli, Italy

The t(4;11)(q21;q23) translocation marks an aggressive acute lymphoblastic leukemia (ALL). The resulting fusion gene encodes the oncoprotein MLL-AF4, which induces expression of genes (*HOXA9*, *MEIS1*) that control cell proliferation. Human AF4 is part of a protein complex that promotes transcription elongation and chromatin remodeling. In t(4;11) ALL, AF4 interacts with MLL-AF4 *(Cancer Cell*2010,17:198-212). We found that 14-3-3θ is a new interactor of AF4 (*Biochem J*2011,438:121-31). *In vitro* binding and FRET assays show 14-3-3θ and AF4 are direct nuclear interactors. In RS4;11 leukemia cells, 14-3-3θ overexpression increases *HOXA9* and *MEIS1* transcription; 14-3-3θ silencing downregulates *HOXA9* and *MEIS1*, reduces cell viability/proliferation, induces apoptosis. ChIP assay shows that 14-3-3θ does not localize on the *HOXA9* promoter; however, it affects the binding of AF4 on the *HOXA9* promoter. These data indicate that 14-3-3θ, via the binding to AF4, contributes an oncogenic-like activity by affecting the MLL-AF4 aberrant activity in leukemia cells. Therefore, 14-3-3θ is a potential therapeutic target in t(4;11) ALL. *Reg Campania DC 11/14;PON01_2589-2012 MIUR;POR Campania FSE2007/2013*

P15.2
ΔN-P63α and TA-P63α exhibit intrinsic differences in transactivation specificities that depend on distinct features of DNA target sites

P. Monti[1], Y. Ciribilli[2], A. Bisio[2], G. Foggetti[1], I. Raimondi[2,3], P. Campomenosi[3,4], P. Menichini[1], A. Inga[2], G. Fronza[1]
[1]Mutagenesis Unit, Istituto di Ricerca e Cura a Carattere Scientifico Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy, [2] Laboratory of Transcriptional Networks, Centre for Integrative Biology, CIBIO, University of Trento, Trento, Italy;, [3] Department of Biotechnology and Life Sciences, DBSV, University of Insubria, Varese, Italy, [4] The Protein Factory, Centro Interuniversitario di Ricerca in Biotecnologie Proteiche, Politecnico di Milano, ICRM-CNR Milano and Università degli Studi dell'Insubria, Varese, Italy

TP63 is a member of the TP53 gene family encoding for different TA and ΔN isoforms. Besides being a master regulator of epithelial development and differentiation, P63 plays important roles in tumorigenesis. P63 isoforms share a central DNA binding domain responsible for binding to sequence-specific response elements (REs). To examine the contribution of RE sequence features to P63-dependent transactivation specificity we took advantage of a yeast functional assay where a single P63 isoform can be expressed and its capacity to activate transcription from isogenic promoter-reporter constructs measured. From the results obtained with P63 proteins on more than 80 different REs, those obtained with P73 and P53 proteins for a selected group of REs, and also from the correlation between yeast- and mammalian-transcription assays, we uncovered that ΔN- and TA-P63α exhibit different transactivation specificities. These changes are 1) dependent on specific features of the target RE sequences, 2) not observed with corresponding P73 and P53 proteins, and 3) likely related to intrinsic differences in the oligomeric state and in the cooperative DNA binding between ΔN- and TA-P63α proteins.

P15.3
KLF7 regulates TUBB3 gene expression in ovarian cancer

S. Mozzetti[1], F. Filippetti[1], M. Petrillo[1], S. Prislei[1], G. Raspaglio[1], M. De donato[1], E. Martinelli[1], C. Ferlini[2], G. Scambia[1]
[1]Dept of Obstetric and Gynecology, Catholic University of Sacred Heart, Rome, Italy, [2]Reproductive Tumor Biology Research, Biomedical Lab, Dept of Obstetrics and Gynocology, Danbury Hospital, Danbury, CT, USA

Drug resistance remain an ongoing challenge in ovarian cancer treatment. TUBB3 is a well known marker of chemoresistance and poor outcome in ovarian cancer and the analysis of the regulation of its expression is of remarkable interest. KLF7 binding elements are present on promoter region of TUBB3. KLF7 is a member of Krupper-like factors (KLFs), a family involved in many biological processes, including: proliferation, apoptosis, differentiation, and development. KLFs play also a critical role in the onset and development of numerous tumor types. We demonstrate that KLF7 overexpression increases TUBB3 expression while KLF7 silencing decreases TUBB3 levels. Moreover KLF7 silencing confers taxol sensitivity to ovarian cancer cell lines., Catechins are natural antioxidant flavonoids, which are under active investigation as anticancer therapies in several human malignancies. It was observed that (-)catechin is able to reduce KLF7 expression in adipocytes. We demonstrated that (-) catechin treatment was able to downregulate Klf7 expression in ovarian cancer cell lines, suggesting that KLF7 may represent an attractive therapeutic target in ovarian cancer.

P15.4
Role of the ferritin heavy chain (FHC) in CXCR4/CXCL12 pathway

M. Panebianco, I. Aversa, M. Di Sanzo, A. Di Vito, S. Bulotta, N. Lobello, F.S. Costanzo
Experimental and Clinical Medicine Department, University Magna Græcia of Catanzaro, 88100 Catanzaro

CXCR4, the receptor for the chemokine CXCL12, is involved in critical processes such as leukocyte homing, development of the immune and central nervous systems, human immunodeficiency virus infection and cancer metastasis. It has been demonstrated that the heavy ferritin subunit (FHC) interacts with internalized CXCR4 and interferes with CXCR4/CXCL12 axis. In this work, we have analysed the CXCR4 pathway in SKOV3 cells in which the FHC expression has been knocked-down by using the shRNA silencing technique in comparison with SKOV3 cells transfected with a control shRNA without significant homology to known human mRNAs. We observed that the FHC-silencing is accompanied by a consistent activation of pAKT and pERK and by an increased migration ability, as measured by wound healing assays. The enhanced migration ability of the FHC-silenced cells is inhibited by the addition of AMD3100 peptide, a specific CXCR4 inhibitor. The chemokine receptor-mediated cell migration is critical in inflammation processes and cancer metastasis. Since the FHC expression consistently differs in different tissues and tumor-types, further studies will focus on the relationship among different FHC levels and different cell sensitivity to chemokine stimuli.

P15.5
An atypical ligand for the fourth PDZ domain of Scribble

F. Storino, S. La Tesse, R. Gallo, M. Furchì, M. Frascà, S. Panni

PDZ domains are protein recognition modules involved in several crucial biological processes through their role in assembling functional complexes. The general features of PDZ have been extensively studied in the last few years, showing that their binding pocket is specific to the carbossi-terminal of target proteins, although they can occasionally recognize other regions. The tumor suppressor Scribble contains four PDZ domains that mediate its function. The role of the fourth PDZ is controversial: dissimilar, or specie-specific, ligands have been reported in studies concerning its binding specificity. We have recently shown that this domain has a crucial role in the formation of a complex between Scribble and the Dual Specificity Phosphatase Dusp26. Since no

canonical consensus for PDZ binding is present in the phosphatase, we have analyzed this interaction more in detail by mutagenesis analysis, to highlight which residues of the phosphatase are important for the binding. Finally we show that the C-terminal domain of Scribble may have a regulatory role on this interaction.

P15.6
The transcriptional factor EGR1 regulates the expression of nucleolar proein B23

D. Ponti[1], D. Bastianelli[1], G.C. Bellenchi[2], P. Rosa[1], R. Puca[1], G. Ragona[1], A. Calogero[1]
[1]Dep. of Medico-Surgical Sciences and Biotechnologies , Univ. of Rome Sapienza, Latina, Italy, [2]Istituto di Genetica e Biofisica, CNR, Napoli, Italy

The nucleolus is a multi-domain containing proteins involved in several processes such as ribosome biogenesis, cell cycle control, apoptosis, viral replication and differentiation of stem cells. Our recent results have demonstrated that the transcriptional factor EGR1 localizes in the nucleolus and similar to p53 acts as negative regulator of RNA polymerase I activity (Ponti et al 2014). The access to the nucleolar compartment is generally mediated by B23 that acts as shuttle between cytoplasm/nucleo/nucleolus. To investigate a possible crosstalk between these two proteins we performed confocal analysis and we observed that EGR1 and B23 colocalize in the nucleolus. Furthermore after B23 silencing we shown a reduced nucleolar localization of EGR1 suggesting a possible role of B23 as nucleolar shuttle for EGR1. Moreover we identified a potential binding site for EGR1 on the B23 promoter that was confirmed by transactivation experiments and Chip assay. Interestingly the levels of B23 in the brain of the Egr1 -/- mice were significantly reduced thus supporting the regolative role of EGR1 on B23 expression.

P15.7
ZEB2 gene expression is regulated by the RNA binding protein HuR and correlates with prognosis in ovarian cancer

S. Prislei[1], E. Martinelli[1], M. Petrillo[1], F. Filippetti[1], M. Mariani[3], S. Mozzetti[1], G. Raspaglio[1], G. Zannoni[1,2], C. Ferlini[3], G. Scambia[1]
[1]Dept of Obstetrics and Gynecology, Catholic Univ of the Sacred Heart, Rome, Italy, [2]Department of Pathology, Catholic University of the Sacred Heart, Rome, Italy, [3]Reproductive Tumor Biology Research, Biomedical Lab, Dept of Obstetrics and Gynecology, Danbury Hospital, Danbury, CT, USA

The epithelial-mesenchymal transition (EMT) is a genetic program that controls cell migration in embryonic development and adult tissue homeostasis, while in epithelial tumor cells EMT contributes to the formation of cancer stem cells and metastasis. The transcriptional repressor ZEB2 is a key factor in EMT and its expression is mediated by the miR-200 family. Since miR-200c interplay with the RNA-binding protein HuR in modulation of the ovarian prognostic factor TUBB3 (class III beta-tubulin), we investigated HuR involvement also in modulation of ZEB2 gene expression. HuR binding sites are present in 3'UTR sequence in proximity of miR-200s binding sites and in Hey cells we demonstrated HuR association by pull-down and RIP. HuR silencing decreased the expression of ZEB2 protein, indicating its role as a positive regulator of ZEB2 post-transcriptional modulation. ZEB2 silencing in Hey cells reduced the migration and the anchorage-independent cell growth ability. The analysis of 220 ovarian cancer patients showed that higher levels of ZEB2 mRNA correlates with shorter OS and PFS. Our findings indicated that ZEB2 is an unfavourable factor that may facilitate ovarian cancer progression.

P15.8
New non canonical functions for human dyskerin

I. Stufa[1], V. Belli[2], A. D'Amore[1], N. Matrone[1], M.Furia[1,2]
[1]Dept of Biology, University of Naples Federico II, Italy, [2]Center for Advanced Biomaterials for Healthcare, IIT; Interdisciplinary Research Centre on Biomaterials (CRIB), University of Naples Federico II, Italy

X-linked dyskeratosis is a syndrome characterized by ribosomal defects, telomere shortening, stem cell loss and tumor predisposition. The disease is caused by hypomorphic mutations of *DKC1,*a gene that encodes dyskerin, a nucleolar protein that acts as oncosuppressor and exhibits N- and a C-terminal nuclear localization signals, and a new truncated isoform that lacks the C-terminal signal. While dyskerin acts as pseudouridine synthase and controls multiple cellular processes, the biological roles of the new isoform remain to be established. As a first approach to define these functions, we stably overexpressed the new isoform and analysed in detail its intracellular localization by multiphoton confocal microscopy. These analyses revealed that the truncated isoform is prevalently confined in the cytoplasm and dynamically can co-localize with intracellular vesicles or translocate into the nuclei, being involved in nucleo-cytoplasmic shuttling. These data hint at unexpected non-canonical functions of dyskerin, implying a central role in regulating the signal transduction pathways originating in the ER and traveling to the nucleus or to membrane.

O15.1
PDK1-mediated activation of MRCKα regulates directional cell migration and *lamellipodia* retraction

P. A. Gagliardi[1,2], L. di Blasio[1,2], A. Puliafito[1,2], G. Seano[1,2], R. Sessa[1,2], F. Chianale[1,2], T. Leung[3], F. Bussolino[1,2,4], L. Primo[1,2,4]
[1]Department of Oncology, University of Torino, Turin, Italy, [2]Laboratory of Cell Migration, Candiolo Cancer Institute FPO-IRCCS, Candiolo, Italy, [3]Institute of Molecular and Cell Biology, A-STAR, 61 Biopolis Drive, Singapore, [4]Center for Molecular Systems Biology, Torino

Directional cell migration is of paramount importance in both physiological and pathological processes, such as development, wound healing, immune response, and cancer invasion. Here, we report that 3-phosphoinositide-dependent kinase 1 (PDK1) regulates epithelial directional migration and invasion by binding and activating myotonic dystrophy kinase-related CDC42-binding kinase α (MRCKα). We show that the effect of PDK1 on cell migration does not involve its kinase activity but instead relies on its ability to bind membrane phosphatidylinositol(3,4,5)-trisphosphate. Upon epidermal growth factor (EGF) stimulation, PDK1 and MRCKα colocalize at the cell membrane in *lamellipodia*. We demonstrate that PDK1 positively modulates MRCKα activity and drives its localization within *lamellipodia.* Likewise, the retraction phase of *lamellipodi*a is controlled by PDK1 through an MRCKα-dependent mechanism. In summary, we discovered a functional pathway involving PDK1-mediated activation of MRCKα, which links EGF signaling to myosin contraction and directional migration.

O15.2
The 5'-untranslated region of p16^{INK4a} melanoma tumor suppressor acts as a cellular IRES, controls mRNA translation during hypoxic stress, and is a target of YBX1

A. Bisio[1], E. Latorre[2], V. Andreotti[3], B. Bressac-de Paillerets[4], M. Harland[5], G. Bianchi Scarra[3], P. Ghiorzo[3], R. C. Spitale[6], A. Provenzani[2], A. Inga[1]
[1]Laboratory of Transcriptional Networks, Centre for Integrative Biology, CIBIO, University of Trento, Trento, Italy, [2]Laboratory of Genomic Screening, Centre for Integrative Biology, CIBIO, University of Trento, Trento, Italy, [3]Laboratory of Genetics of Rare Hereditary Cancers, DiMI, University of Genoa, Italy and IRCCS AOU San Martino-IST, Genoa, [4]Service de Génétique, Institut Gustave Roussy, Villejuif, France, [5]Section of Epidemiology and Biostatistics, Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, UK, [6]Department of Pharmaceutical Sciences, University of California, Irvine

Here we report that p16^{INK4a} belongs to the expanding group of genes whose translation is influenced by sequence/structural features of the 5'UTR mRNA that are endowed with cellular Internal Ribosome Entry Site (IRES) activity. The potential for p16^{INK4a} 5'UTR to drive cap-

independent translation was evaluated by dual-luciferase assays using bicistronic vectors. Results of reporters' relative activities coupled to control analyses for actual bicistronic mRNA transcription, indicated that the wild type $p16^{INK4a}$ 5'UTR could stimulate cap-independent translation. Notably, hypoxic stress and the treatment with mTOR inhibitors, enhanced the translation-stimulating property of $p16^{INK4a}$ 5'UTR. RNA immunoprecipitation performed in melanoma-derived SK-Mel-28 and in patients' derived lymphoblastoid cells indicated that YBX1 can bind the wild type $p16^{INK4a}$ mRNA but not a c.-42T>A variant, and increase its translation efficiency, particularly during hypoxic stress. Modulation of YBX1 expression further supported its involvement in $p16^{INK4a}$ cap-independent translation. RNA SHAPE assays revealed local flexibility changes for the c.-42T>A variant at the predicted YBX1 binding site region. Our results indicate that $p16^{INK4a}$ 5'UTR contains a cellular IRES that can enhance mRNA translation efficiency, in part through YBX1.

O15.3

MDM2-mediated degradation of p14ARF: a novel mechanism to control ARF levels in cancer cells

M. Vivo, M. Matarese, V. Calabrò, G. La Mantia, A. Pollice

Dept. Biology, Univ of Naples Federico II MSA

Knowledge of the mechanisms governing the unbalance between tumor suppressors and oncogenes, is critical to understanding the pathogenesis and evolution of cancer. Among the most important tumor suppressors, p14ARF (Alternative Reading Frame) appears to play a major role by sensitizing incipient cancer cells to undergo growth arrest or apoptosis through MDM2/p53 dependent or independent pathways. We now propose an "upturn" in our way to look at the MDM2/ARF relationship as we observed that MDM2 intracellular increase can cause ARF destruction by the proteasome in various cancer cell lines. The effect does not require the ubiquitin ligase activity of MDM2 and preferentially occurs in the cytoplasm. Interestingly, treatment with inhibitors of the PKC (Protein Kinase C) pathway and use of p14ARF phosphorylation mutants indicate that ARF phosphorylation could play a role in MDM2 mediated ARF degradation reinforcing our previous observations that ARF phosphorylation influences its stability and biological activity. Our study uncovers a new potentially important mechanism through which ARF and MDM2 can counterbalance each other during the tumorigenic process.

O15.4

Epigenetic role of the N-MYC/LSD1 complex in Neuroblastoma

M.C. Sorrentino[1], G. Milazzo[2], S. Amente[1], C. Raimondo[1], S. Ambrosio[1], S. Gherardi[2], L. Lania[1], G. Perini[2], B. Majello[1]

[1]Department of Biology, University of Naples 'Federico II', Naples, Italy, [2]Department of Pharmacy and Biotechnology University of Bologna

Childhood Neuroblastoma is the most common solid tumour of infancy, highly refractory to therapy. One of the most powerful prognostic indicators for this disease is the N-Myc gene amplification, which occurs in approximately 25% of Neuroblastomas. N-Myc regulates transcription by binding the E-box sequence. The histone demethylase LSD1 is highly expressed in undifferentiated Neuroblastomas with an adverse outcome. We report that LSD1 form a tight complex with N-Myc recruiting LSD1 on targets genes., Several human Neuroblastoma cell lines were employed to determine the interaction between N-Myc and LSD1, to define the occupancy of the complex on N-Myc targets and to estimate growth rate and cell cycle distribution as a function of LSD1 expression levels or inhibition of LSD1 activity. LSD1 inhibition affects N-Myc functions, by either up-regulating (Ncl) or inhibiting canonical N-Myc target genes (p21). LSD1 inhibition reduces cell proliferation and blocks cells in G1 phase. Our results point to LSD1 as a critical regulator of N-Myc activity, which can be targeted by specific drug inhibitors. These findings pose bases for novel therapeutic approaches to treat advanced Neuroblastomas.

O15.5

YB-1 and ΔNp63α cross-talk in the control of squamous carcinoma cell adhesion and survival

A. Troiano, O. di Martino, I. Schiano Lomoriello, M. Vivo, A. Pollice, G. La Mantia, V. Calabrò

Dept of Biology, Univ. of Naples, Federico II, Italy

Most cutaneous squamous cell carcinomas (SCCs) contain p53 mutations. The presence of p53 mutations in premalignant lesions suggests that they represent early events during tumor progression and additional alterations may be required for SCC development. While SCCs typically lack somatic oncogene-activating mutations, they frequently exhibit high levels of ΔNp63α and Y-box binding 1 (YB-1) oncoproteins. YB-1 knockdown by RNA interference in HaCaT cells leads to a dramatic decrease of ΔNp63α transcript and protein level with consequent apoptosis. In SCC022 cells, instead, YB-1 depletion results in PI3K/Akt signalling activation that enhances ΔNp63α expression and contributes to cell survival. However, PI3K pharmacological inhibition or YB-1 and ΔNp63α double-knockdown by RNAi induced cell detachment and necrotic death which was associated to decreased levels of CD44 cell surface protein. Here we present data unveiling a functional cross-talk between YB-1 and ΔNp63α governing adhesion and survival of squamous carcinoma cells.

16 - Stem Cells, IPS, Cancer Stem Cells

P16.1
Mesenchymal stromal cell secreted sphingosine 1-phosphate exerts a stimulatory effect on skeletal myoblast proliferation.

A. Frati[1], C. Sassoli[2], A. Tani[2], G. Anderloni[1], F. Pierucci[1], F. Matteini[1], F. Chellini[1], S. Zecchi Orlandini[2], L. Formigli[2], E. Meacci[1]
[1]Dept of Experimental and Clinical Biomedical Sciences – Univ. Florence, [2]Dept of Experimental and Clinical Medicine – Univ. Florence

Bone-marrow-derived mesenchymal stromal cells (MSCs) have the potential to significantly contribute to skeletal muscle healing through the secretion of paracrine factors that support proliferation and enhance participation of the endogenous muscle stem cells in the process of repair/regeneration. However, MSC-derived trophic molecules have been poorly characterized. The aim of this study was to investigate paracrine signaling effects of MSCs on skeletal myoblasts. It was found, using a biochemical and morphological approach that sphingosine 1-phosphate (S1P), a natural bioactive lipid exerting a broad range of muscle cell responses, is secreted by MSCs and represents an important factor by which these cells exert their stimulatory effects on C2C12 myoblast and satellite cell proliferation. Finally, we also demonstrated that the myoblast response to MSC-secreted vascular endothelial growth factor (VEGF) involves the release of S1P from C2C12 cells supporting a crosstalk between the two signaling pathways.. Our data may have important implications in the optimization of cell-based strategies to promote skeletal muscle regeneration.

P16.2
Dynamic intracellular localization of natural alkaloids in different human tumor cell lines: a case study with berberine

M. Natali[1], S. Marracci[2], R. Batistoni[2], P. Deri[2], A. Ferrari[3], A.M. Bianucci[3]
[1]Dept Pharmacy, Pisa Univ., Pisa, Italy, [2]Dept Biology, Pisa Univ., Pisa, Italy, [3]Istituto Nazionale per la Scienza e Tecnologia dei Materiali, Florence, Italy

Berberine is an alkaloid present in plant extracts and has a history of use in traditional Chinese medicine. Because of its ability to arrest the cell cycle and cause apoptosis of several malignant cell lines, it has received attention as a potential anticancer therapeutic agent. To investigate pathways where berberine could play anticancer role, we started to analyze intracellular localization pattern of this alkaloid in different human cell lines: MIA PaCa-2 from pancreatic carcinoma, U343 from glioblastoma multiform. Human dermal fibroblasts (HDF) were used as non-tumor control. Berberine presents natural green fluorescence, which allows identification of the intracellular site of accumulation in living cells. Cells incubated in berberine at different micro-molar concentrations for different times show an intracellular distribution specifically *related* to *dose and time* of *treatment*in the analyzed cell lines. At lower doses and times, the alkaloid is mainly detected in cytoplasmic perinuclear structures; at higher doses and times, it localizes in nuclei. Berberine dose-dependent cell death was observed. Further experiments using specific intracellular markers are in progress.

P16.3
SOX2 is critical for human melanoma initiating cells

S. Pietrobono[1], R. Santini[1], S. Pandolfi[1], V. Montagnani[1], J.Y. Penachioni[1], M.C. Vinci[1,2], S.G. Conticello[3], B. Stecca[1]
[1]Laboratory of Tumor Cell Biology, Core Research Laboratory—Istituto Toscano Tumori (CRL-ITT), Florence, Italy, [2]Current address: Centro Cardiologico Monzino, Milano, Italy, [3]Laboratory of Molecular Mechanisms of Oncogenesis, Core Research Laboratory—Istituto Toscano Tumori (CRL-ITT), Florence, Italy

Melanoma is an aggressive form of skin cancer due to its high invasivity and resistance to chemotherapy. Recent findings show that the Hedgehog (HH) signaling drives self-renewal and tumorigenicity of melanoma initiating cells (MICs). Here we address the role of SOX2 in melanoma cells and MICs and its interplay with HH signaling., We find that HH pathway regulates SOX2 expression and that the downstream effectors GLI1 and GLI2 bind to SOX2 promoter. We demonstrate that SOX2 acts as a mediator of HH signaling in controlling melanoma cell growth and MIC self-renewal., We show that SOX2 is highly expressed in a population enriched for cancer stem cells in patient-derived melanomas. SOX2 knockdown decreases self-renewal of melanoma spheres and of $ALDH^{high}$ MICs. SOX2 silencing also inhibits cell growth and promotes apoptosis. Depletion of SOX2 strongly impairs tumor growth and tumor initiating capability of $ALDH^{high}$ MICs upon xenotransplantation., Our data identify SOX2 as a critical factor for the survival of MICs and an important mediator of HH signaling in melanoma. These findings could provide the basis for novel therapeutic strategies in melanoma treatment based on SOX2 inhibition.

P16.4
Sialidase NEU4 inhibition reduces glioblastoma stem cell renewal

F. Testa[1], I. Silvestri[1], R. Zappasodi[2], C.W. Cairo[3], Y. Zhang[3], R. Galli[4], M. Di Nicola[2], B. Venerando[1], C. Tringali[1]
[1]Department of Medical Biotechnology and Translational Medicine, University of Milan, Segrate (Milan), Italy, [2]Medical Oncology Department, Fondazione IRCCS Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy, [3]Alberta Glycomics Center, Department of Chemistry, University of Alberta, Edmonton Alberta T6G 2G2, Canada, [4]Neural Stem Cell Biology Unit, Division of Regenerative Medicine Stem Cells and Gene Therapy, San Raffaele Scientific Institute, Milan, Italy

Sialidase NEU4 has emerged as a regulator of neuronal differentiation and its over-expression has been showed to promote the acquisition of a stem cell-like phenotype in neuroblastoma cells.[1] We demonstrated that glioblastoma stem cells (GSCs) isolated from glioblastoma (GBM) cell lines and patients' specimens as neurospheres are marked by the up-regulation of NEU4; in contrast, the expression of NEU4 is very low in non-neurosphere differentiated GBM cells. NEU4 silencing or a chemical inhibition of its catalytic activity induced in GSCs: a) the activation of GSKb3, with the consequent inhibition of Sonic Hedgehog and Wnt/b-catenin pathways; b) the decrease of the stem cell-like gene expression and marker signatures, *i.e.* NANOG, OCT-4, SOX-2, CD133 expression, ganglioside GD3 synthesis, and protein glycosylation profile; c) a significant decrease in survival. Instead, increased NEU4 activity and expression induced in the more differentiated GBM cells by the NEU4 agonist thymoquinone increased the expression of OCT-4 and GLI-1. Thus, NEU4 expression and activity appeared to be strictly connected with their survival properties., Tringali C et al (2012) Int J Cancer 131(8):1768-78

P16.5
Human osteosarcoma 3AB-OS cancer stem cells is a model to study microRNA-29b-1 involvement in self-renewal and fate decisions of stem cells

R. Di Fiore[1], R. Drago-Ferrante[1], F. Pentimalli[2], D. Di Marzo[2], I.M. Forte[2], R. Vento[1]
[1]Laboratory of Biochemistry, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Polyclinic, Palermo, Italy, [2]INT-CROM, 'Pascale Foundation', National Cancer Institute - Cancer Research Center, Mercogliano, Avellino, Italy

A successful cure of cancer requires cancer stem cells (CSCs) eradication. Recently, using the human osteosarcoma MG63 cells, we produced a pluripotent CSC line (3AB-OS) where we showed a

potent down-regulation of miR-29b-1. This is a component of the microRNA-29 family frequently down-regulated in osteosarcoma (OS), the most common form of childhood cancer with a potent metastasizing potential. Here, after stable transfection of 3AB-OS cells with miR-29b-1, we investigated the changes in their proliferation, sarcosphere-formation ability, clonogenic growth, chemosensitivity, migration and invasiveness. miR-29b-1 overexpression markedly reduced cell growth, sarcosphere/colony formation even sensitizing the cells to anticancer drugs. We also analyzed potential miR-29b target genes as cell cycle/apoptosis regulators (N-Myc, CCND2, E2F1 and E2F2, Bcl-2 and IAP-2) and stemness markers (CD133, Oct3/4, Sox2 and Nanog). We showed that miR-29b-1 potently down-regulated the expression of these targets. We suggest that identification of CSC-related miRNAs would provide information for understanding CSCs role and that using miR-29b-1 as a therapeutic agent might offer benefits for OS cure.

O16.1
Diacylglycerol kinase alpha contributes to tumorigenicity and invasiveness of cancer stem cells

D. Capello[1], M. Savino[1], V. Bettio[1], E. Agosti[1], S. Faletti[1], G. Baldanzi[1], G. Pelicci[1], M. Todaro[2] F. Sinigaglia[1], G. Stassi[2], A. Graziani[1]
[1]Dept. of Translational Medicine, University of Piemonte Orientale, Novara, Italy, [2]Dept. of Surgical and Oncological Sciences, University of Palermo, Palermo, Italy

Activation of Diacylglycerol Kinase-alpha (DGKa) by growth factors, oncogenes, chemokines and extracellular matrix mediates their ability to stimulate proliferation, migration and invasion in neoplastic cells. Moreover DGKα inactivation impairs tumor growth and viability. Nonetheless the mechanisms that underlie the contribution of DGKα to tumurigenesis have not been clarify. We aimed to investigate the function of DGKα in colon, breast and glioblastoma cancer stem cells (CSCs). By siRNA-mediated silencing and pharmacological inhibition, we showed that DGKa is dispensable for CSCs growth, but is required for EMT and MET transition in 2D and 3D culture, resistance to chemotherapics and invasion. Consistently, DGKa silencing results in strong reduction of the expression of IL4, which sustains chemoresistance, and alters the expression of Twist and Snail, which coordinate EMT/MET transition and the invasive phenotype of cancer cells. Furthermore, xenograft of breast CSCs lacking DGKa featured a strong defect of primary tumor growth. In summary these data provide the first demonstration that DGKa is required for cancer stem cell properties necessary for tumor growth.

O16.2
Role of adenosine receptors in survival and differentiation of glioblastoma stem cells

S. Daniele, E. Zappelli, L. Natali, ML. Trincavelli, C. Martini
Dept Pharmacy, Univ., Pisa, Italy

Glioblastoma Multiforme (GBM) is a highly aggressive brain tumor characterized by increased proliferation and resistance to chemo- and radiotherapy. Recent research hypothesize that much of the therapy resistance and recurrence of the tumor may rest within a small population of cells, known as "cancer stem cells" (CSCs). Extracellular purines have been implicated in several aspects of GBM biology: they control proliferation and migration of tumor cells and affect self-renewal and differentiation properties of CSCs. Herein, we investigated the role of different adenosine receptor (AR) subtypes in survival/differentiation of CSCs isolated from human GBM. Stimulation of A_1 and A_{2B} ARs showed a prominent anti-proliferative/pro-apoptotic effect on CSCs. Of note, the A_1AR agonist was also able to induce CSC differentiation toward a glial phenotype. Most importantly, AR agonists sensitize CSCs to the genotoxic action of temozolomide (TMZ) and make these effects time-prolonged trough different mechanisms: i) potentiating TMZ pro-apoptotic effects in the case of A_{2B}AR; ii) driving cells to a differentiated phenotype more sensitive to TMZ, in the case of A_1AR. These results suggests that targeting of membrane purinergic receptors, followed by the use of TMZ, could be a novel approach in the development of therapeutic strategies against GMB recurrences.

O16.3
Role of Polycomb/HIF/VEGF pathway in the etiology of mesothelioma

E. Paolicchi[1], O. Melaiu[1], E. Barone[1], L. Mutti[2], F. Gemignani[1], S. Landi[1]
[1]Department of Biology, Division of Genetics, University of Pisa, Pisa, Italy, [2]Laboratory of Clinical Oncology, Vercelli National Health Trust, Vercelli, Italy

HIF-1α and VEGF overexpression, leads to Polycomb Group Genes (PcG) overexpression, suggesting that PcG are components involved in hypoxia and angiogenesis. We hypothesized that pro-tumorigenic effects of HIF-1α and VEGF could be moderated by altering the expression of PcG in malignant mesothelioma cell lines (MMCL). We investigate Mesothelin (MSLN), PcG (EZH2 and BMI-1), VEGF and HIF-1α mRNA expression in 6 MMCL and the modulation of their expression by 1% of O_2. The CSCs spheroids formation and their phenotypic characteristics were studied for each CL grown in hypoxia after 24h, 48h and 72h. Future studies will be focused on the inhibition of PcG with siRNA. Our data suggest that hypoxia strongly induces the expression of PcG and VEGF regulated by HIF-1α in MMCL, whereas Met5A showed a decrease of these onco-genes expression. After transient hypoxia, MSLN levels remained high in MMCL. Furthermore, hypoxia increased the number of CSCs spheroids in each MMCL, including Met5A. This study demonstrates that the tumor microenvironment play a pivotal role in the activation of Polycomb/HIF/VEGF pathway, suggesting new options for the design of really targeted trials in MM patients.

O16.4
Allosteric modulation of A_{2B} adenosine receptors favours mesenchymal stem cell differentiation to osteoblasts

M.L. Trincavelli[1], S. Daniele[1], C. Giacomelli[1], S. Taliani[1], F. Da Settimo[1], G. Greco[2], B. Cosimelli[2], E. Novellino[2], C. Martini[1]
[1]Dept Pharmacy, Univ. Pisa, Italy, [2]Dept of Pharmacy, Federico II Univ., Naples, Italy

Purine receptors, and in particular A_{2B} adenosine receptors (ARs), have been recently proposed to control osteoblast differentiation, highlighting this receptor as a new target in bone diseases. In the present study we characterized a new indole-derivative (KI-7) as the first positive allosteric modulator (PAM) of the human A_{2B} AR in mesenchymal stem cells (MSCs), and we investigated the potential activity of this compound as osteogenic agent. KI-7 was able to increase the effects of A_{2B}AR orthosteric agonists on the expression of osteogenic markers and on osteoblast mineralization. In the early phase of differentiation program, KI-7 significantly potentiated physiological and A_{2B} agonist-mediated down-regulation of IL-6 release. Conversely, during the late stage of differentiation, when most of the cells have an osteoblast phenotype, KI-7 caused a sustained raise in IL-6 levels and an improvement in osteoblast viability. These data suggest that positive allosteric modulation of A_{2B} AR not only favours MSC commitment to osteoblasts, but also ensures a greater survival of mature osteoblasts. Our study paves the way for a therapeutic use of selective positive allosteric modulators of A_{2B} AR in the control of osteoblast differentiation, bone formation and fracture repair.

17 - Immunology and Host-Pathogen Interaction

P17.1
Anti-*Pseudomonal* activity of the amphibian antimicrobial peptide Esculentin(1-21) and plausible mode of action

V. Luca, F. Cappiello, B. Casciaro, M.L. Mangoni
Dept. of Biochemical Sciences "A. Rossi Fanelli", Sapienza University of Rome, Rome, Italy

The formation of *Pseudomonas*biofilms is very common in the lungs of cystic fibrosis patients with chronic infections. The colonization of the respiratory tract by this pathogen usually starts with the tissue adhesion of non-mucoid and motile strains. Subsequently they evolve a mucoid phenotype forming sessile communities, with a protective layer around the cells that confers more resistance to antibiotic therapy. Conventional antibiotics are frequently ineffective mainly because of their undesiderable side effects, emergence of resistant strains or because their lacking activity against pathogenic forms. Due to these reasons, novel anti-infective agents are of great interest to the medical community. Here we report on the potent activity and membrane-perturbing effects of the amphibian antimicrobial peptide esculentin(1-21), on both the free-living and sessile forms of *P. aeruginosa*, as a possible mechanism for biofilm disruption. Moreover Esc(1-21) does not induce resistant strains *in vitro* after multiple exposure to the peptide. Overall, this peptide is a promising template for the generation of new antibiotic formulations to advance care of infections caused by *P. aeruginosa*.

P17.2
Pseudomonas aeruginosa adaptation to the host as a strategy to evade immune system responses

I. Paciello[1], L. Curcurù[1], N. I. Lorè[2], , L. Lembo Fazio[1], C. Cigana[2], A. Bragonzi[2], M. Chamaillard[3], A. Allaoui[4], M. L. Bernardini[1]
[1]Dipartimento di Biologia e Biotecnologie "C. Darwin" Sapienza-Università di Roma, Roma, [2]Infections and Cystic Fibrosis Unit, San Raffaele Scientific Institute, Milano, [3] Centre d'Infection et Immunité de Lille, Institut Pasteur de Lille, Francia, [4]Laboratoire de Bactériologie Moléculaire, ULB, Faculté de Medicine, Bruxelles, Belgio

Recurrent and persistent airway infections with *Pseudomonas aeruginosa* (PA) are common in Cistic Fibrosis (CF) patients and account for severe inflammation and lung damages. Interaction of PA with lung tissue triggers the innate defense mechanism of inflammasome, characterized by IPAF-mediated caspase-1 activation, IL-1β and IL-18 release and pyroptosis of infected cells. To evaluate PA adaptation during interaction with the host, three sequential clinical strains of PA, isolated at different stages of infection (acute and chronic) from the same CF patient were analyzed. All clinical strains are able to trigger the inflammasome platform, but PA isolated from acute infection showed a higher percentage of pyroptosis and a higher IPAF-independent IL-1β release than chronic strains. Modification in LPS and in T3SS underline this process. In fact, during the passage from acute to chronic infection, PA modifies its structures to modulate the inflammasome assembly. These results show a network of inflammasome activation where various platforms are activated. This mechanism optimizes the chances of PA to establish a chronic proliferation niche in the host by evading the immune strategies.

P17.3
Sensitivity to infections of four silkworm *Bombyx mori* strains with different geographical origin

O. Romoli[1], A. Bozzato[1], A. Saviane[2], S. Alberghini[3], P. D'Antona[4], G. Tettamanti[4], A.Squartini[3], S. Cappellozza[2], F. Sandrelli[1]
[1]Dipartimento di Biologia, Università di Padova, Padova, [2] CRA-API, Unità di Ricerca di Apicoltura e Bachicoltura, sede di Padova, Padova, [3]Dipartimento di Agronomia, Animali, Alimenti, Risorse Naturali e Ambiente, Università di Padova, Padova, [4] Dipartimento di Biotecnologie e Scienze della Vita, Università dell'Insubria, Varese

The silkworm *Bombyx mori* is an important organism for the economical and biotechnological value. Several silkworm strains exist and can be grouped in four geographical types, characterised by a different silk production and pathogen resistance. The main effectors of *B. mori*innate immune response are the antimicrobial peptides (AMPs), 21 small peptides involved in the defence against microorganism attack., We evaluated whether the different sensitivity to infections of four *B.mori* strains, with distinct geographical origin and silk productivity, is due to a diverse efficiency in the AMP-mediated immune response. We performed 24 h oral infection experiments with the Gram positive *Enterococcus mundtii* or the Gram negative *Serratia marcescens*. After exposure at the beginning of the 5th larval instar, each *B. mori* strain was followed till the adult stage, determining the survival curve and the AMP induction response. Differences in the evaluated parameters were found among the four *B. mori* strains. Moreover, we identified specific polymorphisms in AMP coding sequences of the four *B. mori* strains. Currently, we are exploring the functional meaning of the detected modifications.

O17.1
Inhibition of Diacylglycerol kinase alpha rescues TCR-induced diacylglycerol signaling and restimulation induced cell death in XLP lymphocytes

G. Baldanzi[1], R. Das[2], V. Malacarne[1], S. Larens[3], E. Ruffo[1], L. Patrussi[4], P. Schwartzberg[5], C. Wuelfing[6], T. C. Baldari[4], I. Rubio[7], K. Nichols[2], A. Snow[3,*], A. Graziani[1]
[1]Dep. Traslational Medicine, University of Piemonte Orientale, 28100 Novara, Italy, [2]Div. of Oncology; Children's Hospital of Philadelphia; Philadelphia, PA USA, [3]Dep. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD USA, [4]Department of Life Sciences, University of Siena, Siena, Italy, [5]Genetic Disease Research Branch NHGR, National Institutes of Health Bethesda, MD USA, [6]School of Cellular and Molecular Medicine, University of Bristol, Bristol, UK, [7]Biomolecular Photonics Research Group, University Hospital Jena, Jena, Germany

XLP-1 is a congenital hematological disorder, leading to a severe lymphocytosis following EBV infection. Lymphocyte expansion is due to defective clearance of EBV+ cells and to T cell resistance to re-stimulation induced cell death (RICD)., XLP-1 is due to loss of function of SAP protein adaptor, which contributes to SLAM family receptors and TCR signaling. In particular, SAP deficiency impairs the inhibition of Diacylglycerol kinase alpha (DGKα) upon TCR triggering, leading to increased diacylglycerol metabolization and decreased TCR signaling to Ras and PKCθ., Inhere we show that DGKα silencing/pharmacological inhibition restore diacylglycerol polarization at the immune synapse as well as downstream signaling in SAP deficient cells. Moreover, DGKα knockdown reestablishes expression of the pro-apoptotic genes Nur77 and Nor1, restoring RICD in SAP silenced cells but also in XLP derived lymphocytes. In vivo, DGK inhibition decreases CD8+ expansion and INFγ production after SAP-/- mice infection with LCMV, an animal model of XLP., These data underscore the relevance of SAP-DGKα interaction for immune homeostasis and suggest DGKα as a putative pharmacological target for XLP treatment.,

O17.2
Shigella flexneri induced cell death in human monocyte derived dendritic cells

A-K. Hermansson[1], L. Lembo Fazio[1], E. De Stefano[1], G. Rossi[2], M. L. Bernardini[1]
[1]Dep of Biology and Biotechnology "Charles Darwin", Sapienza Univ. of Rome, Italy, [2]Faculty of Veterinary Medicine, Univ. of Camerino, Italy

Adaptive immune response to *Shigella* is generally poor. Dendritic cells (DCs) are a key element in adaptive response induction, but little is known about the interaction between DCs and *Shigella*., We infected

human monocyte derived DCs with the *S. flexneri* 5 strains M90T (virulent) and BS176 (avirulent). Both strains were internalized by DCs to a similar degree, but M90T induced cell death, as seen through 1) a reduced number of cells compared to not infected cells and cells infected with BS176, 2) a higher release of lactate dehydrogenase and 3) an increased proportion of propidium iodine stained cells. Interleukin (IL) 6 release was similar, but IL-1β release was higher from M90T infected cells. Using a chloroquine resistance assay we found that M90T was able to escape from the phagosome into the cytosol, but not BS176. This result was confirmed by electron microscopy, where M90T was found in the cytosol and BS176 in vacuols., Conclusion: *Shigella flexneri* is capable of escaping from the phagosome in DCs, and causes rapid cell death with pyroptotic signs. This cell death could be an important contributing cause of the defective adaptive immune response after *Shigella* infection.

O17.3
Molecular characterization of a *Candida orthopsilosis* putative adhesin

L. Lombardi, M. Zoppo, C. Rizzato, A. Bertini, S. Senesi, A. Tavanti
Department of Biology, University of Pisa, Italy

Adhesion to biotic surfaces is a crucial step during the early stages of candidiasis. Little is known on the adhesion genes of the opportunistic pathogen *C. orthopsilosis*. *In silico* analysis of the genomic sequence of *C. orthopsilosis* identified 2 putative *ALS*-like sequences and one incomplete ORF (*CORT0B00800*). *CORT0B00800* sequencing led us to the identification of common *ALS* features in the C-terminal end of the hypothetical Als protein. Real-time RT-PCR confirmed that all three genes are expressed under normal growth conditions, supporting the view that *CORT0B00800*is an adhesin. Tridimensional folding of the N-terminal domain has been modelled *in silico*, resulting in 2 Ig-like domains forming a peptide binding cavity (PBC), similar to what was recently described for *C. albicans ALS3*. In addition, PCR amplification and sequencing of *CORT0B00800*highlighted a large allelic variability between clinical isolates with different adhesive properties, based on different number of tandem repeats, as well as the presence of two amino acidic substitutions in the proximity of the PBC.

O17.4
ApoE-AP, a new human antimicrobial peptide with promising anti-inflammatory properties

V. Sgambati, K. Pane, A. Zanfardino, L. Durante, V. Cafaro, M. Varcamonti, E. Notomista, E. Pizzo
Department of Biology, Federico II University, Naples, Italy

Until today the presence of an antimicrobial peptide in apolipoprotein E was described by Dobson et al (2006). Nevertheless this short 9 residue-long peptide, related to receptor binding region of ApoE, showed a very weak antimicrobial activity. We have recently identified in ApoE, by a bioinformatic method developed by our group, a new 18 residue-long peptide (ApoE-AP). Cytotoxic assays have revealed that ApoE-AP, obtained as recombinant product, shows a high bactericidal activity on several Gram positive and Gram negative bacteria while it is not toxic on human cells.Circular dichroism studies performed in different environments, as membrane-mimicking agents (TFE and SDS), LPS and alginate show that ApoE-AP tends to adopt α-helix conformations in presence of TFE and SDS while it remains soluble in presence of LPS and alginate. Latter data indicate that binding to LPS or to capsular polysaccharides as alginate are not necessary for antimicrobial efficacy of ApoE-AP. Moreover preliminary results by qRT-PCR indicate that ApoE-AP is able to mitigate the expression of some pro-inflammatory factors in human keratinocytes treated with *E.coli* LPS.

O17.5
Targeted gene disruption to investigate the role of *Candida parapsilosis* putative adhesins

M. Zoppo, A. Bertini, L. Lombardi, C. Rizzato, S. Senesi, A. Tavanti
Department of Biology, University of Pisa, Italy

Candida parapsilosis is an important nosocomial pathogen that is responsible for both superficial and systemic candidiasis. The aim of this study was to investigate the role played by 5 potential homologues of *C. albicans* agglutinin-like sequence proteins (Als) in *C. parapsilosis* adhesion to host surfaces (*CPAR2_404800, _404770, _404780, _404790, _500660*). Site specific mutation of the coding sequence of *ALS*-like genes was selected as a tool to dissect their role in the adhesion process. Two independent lines of *CPAR2_404800* heterozygous and null mutants were obtained in *C. parapsilosis* ATCC 22019 genetic background. Mutant strains were characterized for their ability to adhere to human buccal cells. Our results indicated that this gene plays an important role in the adhesion to the human mucosa. The same strategy was then used to disrupt a second putative adhesin (*CPAR2_500660*) in both wild type (ATCC22019) and Δ*cpar2*_404800/*Δcpar2*_404800 KO strain background, to generate single *CPAR2_500660* and double *CPAR2_404800/CPAR2_500660* mutants. Characterization of the mutant panel will help to shed light into the molecular mechanisms involved in *C. parapsilosis* adhesion process.

18 - Plant Development and Disease

P18.1
NMR investigation of random coil regions: clues to assess the different biological activity of two cerato platanin family members

F. Baroni[1], L. Pazzagli[2], S. Luti[2], A. Scala[3], F. Martellini[2], L. Franzoni[1], T. A. Pertinhez[4], A. Spisni[5]
[1]Department of Biomedical, Biotechnological and Translational Sciences (S.Bi.Bi.T.), University of Parma, [2]Department of Biomedical Sciences, University of Florence, Italy, [3]Department of Agricultural Biotechnologies, University of Florence, Italy, [4]C.I.M. Laboratory – Technopole Parma, University of Parma, Italy, [5]Department of Surgical Sciences, University of Parma, Italy

Plant pathogenic fungi secrete several non-catalytic proteins involved in various aspects of the pathogenesis process. Amongst these, cerato-platanin (CP) was first identified and characterized as a PAMP. Aim of the present investigation is to define the basis of the different biological activity between CP and the orthologous protein Pop1, in fact, though both CP and Pop1 are host defense inducers, Pop1 shows a weaker defense induction capacity than CP. Analysis of Pop1 and CP structures and dynamics indicated differences between the two proteins mainly located in the random coil region. Interestingly, this region was proposed to have an important role in oligosaccharides binding and in necrosis induction of leaves' cells; therefore, the different pattern of residues' interactions might be the leading cause of their diverse biological activity. To address this hypothesis we have performed NMR experiments in the course of a titration of both Pop1 and CP with oligosaccharides. We expect that our results, besides providing new hints on the molecular mechanisms operating in plants induced resistance, will contribute to reach the major goal of environment protection.

P18.2
Characterization of cell wall traits of spikes from wheat lines resistant and susceptible to *Fusarium graminearum*

V. Lionetti[1], E. Fabri[1], A. Giancaspro[2], S.L. Giove[2], N. Reem[3] , O.A. Zabotina[3], A. Gadaleta[2], A. Blanco[2], D. Bellincampi[1]
[1]Dipartimento di Biologia e Biotecnologie "Charles Darwin", Sapienza Università di Roma, Rome, Italy, [2] Department of Soil, Plant and Food Science (DiSSPA), University of Bari "Aldo Moro", Via G. Amendola 165/A - 70126, Bari, Italy, [3] Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa 50011

Fusarium graminearum, one of the causal agents of Fusarium Head Blight (FHB), preferentially infects wheat spikelets and leads to severe losses in yield and grain quality due to the production of mycotoxins which are harmful to human and livestock. Different traits for FHB resistance in wheat were identified for common wheat (*Triticum aestivum L.*), while the sources of FHB resistance in durum wheat (*Triticum turgidum* ssp. Durum), one of the cereals most susceptible to *F. graminearum* infection, have not been found. New lines of evidence indicate that content and composition of cell wall polymers affect the susceptibility of cell wall to cell wall degrading enzymes and can play a role in the outcome of host-pathogen interactions. The objective of our research is to identify cell wall biochemical traits contributing to the *Fusariosis* resistance to be transferred from common wheat to the more susceptible durum wheat.

P18.3
The phytotoxin fusicoccin is a general regulator of the interaction of 14-3-3 proteins with their protein targets

L. Camoni[1], L. Cervoni[2], F. Cutruzzolà[2], C. Di Lucente[1], G. Janson[2], A. Paiardini[2], S. Pascarella[2], S. Rinaldo[2], S. Visconti[1], P. Aducci[1]
[1]Department of Biology, University of Rome "Tor Vergata", Italy, [2]Department of Biochemical Sciences, Sapienza University of Rome, Italy

Fusicoccin (FC), a phytotoxin produced by the fungus *Phomopsis amygdali,* affects several processes in plants, as a consequence of H^+-ATPase activation. FC stabilizes 14-3-3 interaction with the H^+-ATPase, thus activating the enzyme. The 14-3-3 binding site (YpTV-COOH, designed as mode III) considerably differs to those present in the majority of 14-3-3 clients (mode I and II). This feature explains why FC selectively stabilizes the 14-3-3/H^+-ATPase interaction., However, a number of 14-3-3 targets with mode III motifs has been recently identified both in plants and animals. Structure similarity between the H^+-ATPase and other targets makes conceivable that other 14-3-3 interactions could be stabilized by FC. Accordingly, we recently demonstrated FC ability to stabilize 14-3-3 association to human platelet glycoprotein GPIbα. This finding proposes FC as a drug-like molecule exploitable to control physiological processes where 14-3-3s take part., Here we show that FC stabilizes 14-3-3 interaction to plant and animal targets with a mode III motif. ITC analysis showed FC ability to stimulate 14-3-3 association to different peptides. Moreover, molecular docking studies provided the structural rationale for FC effect. Our study proposes FC as a promising tool to control cellular processes regulated by 14-3-3s, opening new perspectives on its potential pharmacological applications.

P18.4
Pectic enzymes in the oomycetes pathogenesis

C. Caprari[1],M. Maistrou[1], J. Pham[2], E. Huitema[2]
[1]Dept. of Bioscience and Territory, Univ. of Molise, Pesche (IS), Italy, [2]Division of Plant Sciences, Univ. of Dundee, Invergowrie, Dundee, United Kingdom

The plant cell wall is a structural barrier to microbes, composed of a network of polysaccharides such as cellulose, hemicellulose and pectin. During infection, the host cell wall must be degraded, a feat that is accomplished through an array of microbe-encoded Cell Wall Degrading Enzymes (CWDE). The polygalacturonases (PGs) are among the first CWDE secreted by pathogens to facilitate invasion, release of nutrients and support pathogen growth. PGIPs (polygalacturonase-inhibiting proteins) are plant cell wall proteins that specifically modulate the activity of PGs, and hamper the invasion process by limiting the host tissue colonization. The PG–PGIP interaction retards pectin hydrolysis and favors the accumulation of oligogalacturonides (OGs) leading to plant defense activation. Here we investigate the role of PGs in the oomycetes *Phytophthora nicotianae* and *Phytophthora capsici*on tobacco and tomato plants. We performed phylogenetic analyses to characterise PG families in the two oomycetes and, the infection assays carried out on plants expressing PGIP2 from*P. vulgaris*. Finally we present our latest results studying PG effector function in *Phytophthora* during the infection cycle.

P18.5
Petunia *MAEWEST* and *MAEWESTb* functionally overlap in promoting lateral development of floral organs

E. Costanzo[1,2], P. Morel[1], E. Bertolini[2], M.E. Pè[2], M. Vandenbussche[1]
[1]Laboratory of Reproduction and Development of Plants, UMR5667 (ENS de Lyon, CNRS, INRA, UCBL), Ecole Normale Supérieure de Lyon, Lyon, France, [2]Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy

The Petunia *MAEWEST* (*MAW*) gene has been described as an important player in organ polarity and blade expansion during flower development (Vandenbussche et al., Plant Cell 2009). We identified a second gene from the same MAW/WOX1 clade as *MAW*, called *MAEWESTb* (*MAWb*). Although *mawb* mutants display a WT phenotype, the double mutant *maw mawb* is severely affected in organ polarity and development, compared to WT and *maw* mutants, indicating an important functional overlap. Petals and sepals are extremely narrow and filament-like, giving to the flower an overall star-like phenotype. At the same time, carpels

are shorter and completely unfused, therefore displaying naked and unprotected ovules., To better understand the function of *MAW/MAWb* genes, we performed a NGS based transcriptome analysis on WT, *maw*, and *maw mawb*double mutants. The obtained data will help us to position *MAW/MAWb* function in the known regulatory gene networks controlling organ development and polarity.

P18.6
Assessment of antitumor and antimutagenic activity of saponin fractions from *Astragalus verrucosus*

M.S. Facioni[1], A. De Nittis[1], J. Soares[2], S. Gomes[2], A. Bertoli[3], L. Pistelli[3], L. Saraiva[2], R. Scarpato[1]
[1]Dept Biology, Pisa Univ., Pisa, Italy, [2]Dept Biological Sciences, Porto Univ., Porto, Portugal, [3]Dept Pharmacy, Pisa Univ., Pisa, Italy

In the last decades, natural compounds have attracted considerable attention as cancer chemo-preventive agents, exhibiting many beneficial effects on human health. Among their biological activities, natural products can suppress the growth of established tumors and to modulate apoptosis. To date, saponins isolated from *Astragalus verrucosus*, a perennial herb located in a restricted area of Sardinia, have been poorly studied. The aim of this study was to test the antimutagenic and antitumor activity of several fractionated plant extracts from *A. verrucosus*. For that, these saponin fractions were previously investigated in human lymphocytes for the inhibition of genotoxic effects induced by different anticancer drugs. All the tested extracts exhibited significant antimutagenic activity. Furthermore, their antitumor effect were analysed in a human colon adenocarcinoma cell line. Our results indicated that one of these extracts strongly inhibited the proliferation of this tumor cells with a GI_{50} (concentration that causes 50% growth inhibition) value of 7.92 ± 0.3 µg/mL. Further studies are underway in order to elucidate the mechanism of action of the most promising extract.

P18.7
Characterization of Arabidopsis LysM receptors involved in plant innate immunity

C. Paparella, Daniel V. Savatin, L. Marti, G. De Lorenzo, S. Ferrari
Dipartimento di Biologia e Biotecnologie "Charles Darwin", Sapienza Università di Roma, Rome, Italy

Upon perception of microbe-associated molecular patterns (MAMPs), plants activate an immune response that contributes to resistance against infections. Receptor-like kinases containing one or more lysin motifs (LysM), called LYK proteins, mediate the perception of oligosaccharidic MAMPs, such as chitin and peptidoglycans. Five putative LYKs are encoded by the genome of Arabidopsis thaliana; the function of two of these (LYK1/AtCERK1 and LYK4) in chitin perception has been established, whereas the role of the other three members is unknown. Using reverse genetics, we have found that AtLYK3 negatively regulates the expression of defense genes and resistance to pathogens. Plants lacking a functional AtLYK3 show reduced sensitivity to the hormone abscisic acid (ABA), previously shown to have a negative role in resistance against some pathogens. This suggests that AtLYK3 is important for the cross talk between signalling pathways activated by ABA and pathogens. Additional studies are under way to elucidate the function of the other members of the Arabidopsis LYK family., This work was supported by the European Reearch Council (ERC Advanced Grant 233083)

P18.8
Has *Trichoderma harzianum* 6776 beneficial effects on different cultivation system?

L. Fiorini[1], L Guglielminetti[1], L. Mariotti[1], M. Curadi[1], G.Puntoni[1], A. Scartazza[2], P. Picciarelli[1], S. Sarrocco[1], G. Vannacci[1]
[1]DAFE, Univer. of Pisa, Italy, [2]IBAF-CNR, Rome, Italy

*Trichoderma harzianum*strain T6776 is a potential beneficial isolate to be employed as inoculant of tomato plants since it can promote plant growth and it is able to protect plants against soil-borne and air-borne pathogens., We evaluated the beneficial effects of T6776 in two cultivation systems: a peat based tomato growth substrate and in soilless condition. Different physiological and biochemical parameters have been measured such as growth rate, carbohydrate source-sink portioning, photochemical efficiency of photosystem II, pigment composition and hormonal status of different treated plants. Moreover, we also evaluated the effects of T6776 on PSII under the abiotic stress conditions of salinity and anoxia., This study shows that in both culture conditions T6776 is able to positively affect some of parameters in treated plants, in comparison with the control. Under abiotic stress conditions, T6776 positively influenced plants response, even if more studies are needed to better characterize the associated mechanisms., Moreover, to better understand the genetic potential of T6776, its genome has been recently sequenced and it's now under annotation.

P18.9
The functional characterization of a putative acetylornithine deacetylase of *Arabidopsis thaliana* reveals its role in flowering time and fruit set

F. Martini[1], B. Molesini[1], G. Mennella[2], G. Francese[2], T. Pandolfini[1]
[1]Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134-Verona, Italy, [2]CRA-ORT Agricultural Research Council Research Centre for Horticulture, Via dei Cavalleggeri 25, 84098 – Pontecagnano (SA), Italy

The amino acid ornithine (Orn) is a precursor of polyamines, compounds involved in processes such as fruit set. Two routes have been proposed for Orn biosynthesis: a cyclic pathway controlled by N^2-acetylornithine:N-acetylglutamate acetyltransferase which recycles acetyl group of N^2-acetylornithine on glutamate, and a linear pathway in which Orn is formed from N^2-acetylornithine by the activity of acetylornithine deacetylase (NAOD). The existence of NAOD activity has been demonstrated in few organisms but not in plants. Nevertheless, many putative NAOD-like genes have been identified in plants. In this work, we investigated the role of a putative NAOD gene of *Arabidopsis thaliana*. We obtained two silenced lines and a T-DNA insertional mutant, that showed approximately 90% reduction in NAOD transcript level in comparison with wt. Their phenotypic characterization revealed early and extended flowering as compared with wt plants. In addition, both the mutant and silenced lines showed a number of aborted siliques on the main bolt 5-6 times higher than wt. Orn content was consistently reduced compared with the level in wt suggesting that NAOD regulates Orn level in plant cells.

P18.10
Study of the role of type III LTPs during the symbiotic interaction between *Sinorhizobium meliloti* and *Medicago truncatula*

C. Santi[1], B. Molesini[1], Y. Pii[2], T. Pandolfini[1]
[1]Department of Biotechnologies, University of Verona, Italy, [2] Faculty of Science and Technology, Free University of Bolzano, Italy

Lipid transfer proteins (LTPs) are small basic proteins that constitute a large family characterized by the ability to transfer phospholipids between a donor and an acceptor membrane in *vitro*. Various biological roles for plant LTPs in *vivo* have been proposed, including defense against pathogens and modulation of plant development. The aim of this study is to shed light on the role of three different *Medicago* LTPs in the symbiotic interaction with *S. meliloti*. *MtN5*, a nod factor responsive gene previously identified, is expressed at a very early phase of the symbiosis in epidermal cells and root hairs and has been shown to positively regulate the nodulation process. Stably transformed *MtN5*-silenced and *MtN5*-overespressing plants confirmed the role of this gene in the symbiotic interaction. When inoculated with rhizobia *MtN5*-silenced plants showed a reduced number of invaded primordia in comparison with inoculated wild type plants, whereas *MtN5*-overespressing plants had an increased nodulation capacity. Interestingly, two other putative type III LTPs in the *M. truncatula* genome have been identified. Their expression profiles during the various stages of the symbiosis is under study.

O18.1
The Arabidopsis DAG1 protein plays a key role in regulating hormonal balance during seed development

A. Boccaccini, D. Capauto, R. Lorrai, S. Santopolo, E. Minutello, V. Ruta, P. Costantino, P. Vittorioso
Istituto Pasteur Fondazione Cenci Bolognetti, Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Rome, Italy

Seed germination is controlled by multiple endogenous and environmental factors, integrated to trigger this process at the right time. Two plant hormones play key roles in seed germination: gibberellins (GAs), which have an inductive effect, and abscissic acid (ABA), which inhibits the process. DAG1 is a repressor of this process, and acts downstream of PIL5, which also induces the DELLA gene *GA INSENSITIVE* (*GAI*), which negatively regulates GA metabolism. We recently demonstrated that DAG1 is a fine regulator of GA levels, as it directly regulates the GA biosynthetic gene *AtGA3ox1*, and is itself stabilised by GA at the protein level. GAI indeed cooperates with DAG1 in repressing *AtGA3ox1*, and it directly interacts with DAG1. Moreover, GAI and DAG1 reciprocally regulate their transcription, and their protein regulation suggests a fine-tuning mechanism mediated by GA. More recently we have shown that DAG1 also directly regulates the ABA catabolic gene *CYP707A2*, and that in turn DAG1 is degraded by ABA. In addition, recent data proved that DAG1 plays a key role in regulating the balance between GA and ABA also during seed development and maturation.

O18.2
Members of *AtCuAO* gene family exhibit different tissue-and organ-specific expression patterns during seedling development and distinct responses to hormone or stress treatments

A. Carucci[1], S. Ghuge[1], R. Rodrigues Pousada[2], R. Angelini[1], A. Cona[1]
[1]Dipartimento di Scienze, Università degli Studi "Roma Tre", [2]Dipartimento Scienze della Vita e dall'Ambiente, Università degli Studi dell'Aquila

H_2O_2 derived from amine-oxidase mediated oxidation of polyamines is involved in important processes, such as plant growth and development as well as biotic and abiotic stress responses. Here, our attention has been focused on the analysis of the expression of various copper-containing Amine Oxidase (*AtCuAOs*) and precisely *AtAO1 (At4g14940), AtCuAO1 (At1g62810), AtCuAO2 (At1g31710), AtCuAO7 (At3g43670) and AtCuAO8 (At1g31690),*by both mRNA level investigation and tissue-specific examination of promoter activities. *AtCuAOs*-promoter::*GFP-GUS* activity analysis in different developmental stages revealed that the GUS staining and the GFP signal were detected in different tissues and organs, in particular in the root protoxylem and metaxylem tissue, cortex and epidermis, as well as in the leaf, especially stomata, hydathodes and vascular tissues, suggesting that CuAOs may play a role in differentiation of xylem tissues as well as in plant water balance. Furthermore, expression of different members of *AtCuAO* family is induced by hormones such as methyl-jasmonate, abscisic acid and salicylic acid suggesting a role in development and differentiation influenced by biotic and abiotic stress.

O18.3
PAMP activity of cerato-platanin: a proteomic study

S. Luti[1], A. Caselli[1], L. Mazzoli[1], I. Baccelli[2], L. Lombardi[3], A. Scala[2], L. Pazzagli[1]
[1]Dip. Scienze Biomediche Sperimentali e Cliniche, Univerisità di Firenze, Italia, [2]Dip. Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Università di Firenze, Italia, [3]Dipartimento di Biologia, Università di Pisa, Italia

Cerato-platanin (CP) is a non-catalytic low molecular weight protein characterized by the presence of four cysteine residues to give two intra-molecular disulfide bridges. It is a double ψ-β-barrel protein and is both secreted and is present in the cell wall of hyphae and conidia of *Ceratocystis platani*. CP behaves as PAMP in fact it is able to induce overexpression of MAPKs, production of H_2O_2 and NO_2, programmed cell death, phytoalexin synthesis and restriction of conidia growth. CP is also able to induce the over-expression of defence related genes and to modulate the pathways related to SA, ET and JA., Here we study the PAMP activity of CP by a proteomic approach on Arabidopsis. Leaves of *A. thaliana* were treated with the protein for 8h and subsequently subjected to protein extraction and 2D electrophoresis. As a control, we used water-treated leaves. 1493 spots were resolved and 33 spots showed at least 1,5-fold different protein expression. Twenty of these spots were increased in the treated leaves while thirteen showed decreased accumulation. Differentially expressed spots are being identified by MALDI-TOF to clarify the PAMP activity of CP.

O18.4
Proteomic insights into oligogalacturonide signalling in plant defence and development

B. Mattei, D. Pontiggia, J. Ciarcianelli, G. De Lorenzo
Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Italy

Oligogalacturonides (OGs), fragments derived from the hydrolysis of plant cell wall homogalacturonan by fungal polygalacturonases, are well known damage-associated molecular patterns (DAMPs). In *Arabidopsis thaliana*, perception of OGs triggers an intracellular signaling cascade that initiates defence against pathogens. OGs may also work as regulators of plant growth and development mainly through their antagonism with auxin (IAA). However, most of the mechanism by which the OG signal is transduced is not yet known., In this study, the initial signaling events specifically activated by OGs were investigated by quantitative phosphoproteomics in a membrane-enriched fraction, revealing rapid phosphorylation changes after a 10 min treatment with OGs., The nuclear proteome was analyzed in response to IAA, OGs or a IAA/OG co-treatment, to identify regulatory elements that mediate the OG/IAA antagonism. The observed changes may arise not only from altered expression but also from post-translational modifications, degradation and/or translocation of proteins to different compartments. Interesting candidates in OG/IAA antagonism have been identified and their functional role is under study.

O18.5
Dissecting the role of proline in pollen development

C. Lonoce, E. Mastrorilli, M. Biancucci, I. Recco, R. Mattioli, P. Costantino, M. Trovato
Dept of Biology and Biotechnology, Sapienza University, Rome, Italy

We recently showed that in Arabidopsis the amino acid proline is required for pollen development and fertility, but the precise role of proline in, and the underpinning genetic and molecular basis of, this phenomenon are, as yet, unclear. To fill this gap, we are addressing the problem of the relative contribute of proline synthesis in sporophytic and gametophytic tissues of the anther, with a combination of cellular, genetic and molecular techniques. As a first approach, we are studying the relative expression of the proline synthesis genes *P5CS1*and *P5CS2*, in different anther tissues, by means of in situ hybridization, and GUS analysis. In a complementary genetic approach, we are introgressing the *35S::P5CS1* construct into a *p5cs1 p5cs2/P5CS2* proline-deficent background, to analyze its effects on pollen development and embryo formation and infer hints on the role of proline in maternal and paternal tissues. Finally, with a genetic engineering approach, tapetum specific- and microspore specific-*P5CS2* constructs are being cloned, and will be introduced in *p5cs1 p5cs2/P5CS2* to find out the one/s able to complement the pollen defects of proline-deficient mutants.

19 - Plant Metabolism and Environmental Stress

P19.1
Transcriptomic analysis of light response in *Nannochloropsis gaditana* highlights the basis for specific metabolic remodeling

A. Alboresi[1], G. Perin[1], A. Meneghesso[1], N. Vitulo[2], G. Valle[2], T. Morosinotto[1]
[1]Dipartimento di Biologia, Università di Padova, Padova, 35121, Italy, [2]CRIBI Centro di Biotecnologia, Università di Padova, Padova, 35121, Italy

Seawater oleaginous microalga *Nannochloropsis gaditana* combines a fast growth rate with strong lipid accumulation capacities and it is considered a potential next-generation feedstock for biofuels. Exposure to excess light can enhance lipid accumulation especially if combined with the effect delivered by nutrient deprivation. We carried out large-scale transcriptomic and metabolomic analyses to decipher the acclimation of *N. gaditana* to three different light intensities. Growth was monitored and we evidenced the stimulation of lipid biosynthesis in stronger illumination condition. We compared transcriptomic data to those obtained under nitrogen deficiency, a condition well known to stimulate lipid accumulation. We observed a strong involvement of photosynthesis and chloroplast processes for both stressors, while specific signatures could be associated either to light treatments or to nitrogen depletion. Also concerning lipid biosynthesis, the response to the two conditions was only partially overlapping. We placed the first building blocks for more detailed investigations of the mechanisms underlying lipid production in *Nannochloropsis* species/strains.

P19.2
Understanding cell wall re-modelling during mycorrhizal symbiotic interactions

R. Balestrini[1], F. Sillo[1], F. Cappa[1], A. Faccio[1], P. Bonfante[1], W. Willats[2], J.U. Fangel[2]

Ectomycorrhizal (ECM) fungi have a small set of genes coding for secreted enzymes putatively involved in the degradation of plant cell wall polysaccharides. Within the context of the *Tuber melanosporum* genome sequencing, genes coding for putative plant cell wall degrading enzymes (CWDE) have been identified, and several of them were found to be up-regulated during symbiosis, suggesting a role in plant cell wall degradation to facilitate the progression of the hyphae in the pectin-rich middle lamella, when the fungus develops inside plant tissues. Looking at the arbuscular mycorrhizal (AM) fungi, *Rizophagus irregularis* does not possess degrading enzymes acting on plant cell walls, suggesting that signal molecules released by AM fungi are perceived by the plant cells, which elicit the activation of their own PCW-degrading enzymes. In addition to gene expression analyses, we have employed glycan microarray technology to analyse the impact of fungal colonization on plant cell wall composition in ecto- and AM mycorrhizae. *In situ* immunolabelling experiments, using monoclonal antibodies with specificity for plant cell-wall components, are ongoing with the aim to support glycoarray data.

P19.3
Salts modulate δ[1]-pyrroline-5-carboxylate metabolism in rice

M. Bertazzini[1], S. Giberti, D. Funck[2], G. Forlani[1]
[1]Dept of Life Science and Biotechnology, University of Ferrara, Italy, [2] Dept of Plant Physiology and Biochemistry, University of Konstanz, Germany

Under high nitrogen availability, proline is synthesized mainly from ornithine, which is converted to δ[1]-pyrroline-5-carboxylate (P5C) by an ornithine-δ-aminotransferase. In contrast, under osmotic stress conditions and/or nitrogen starvation P5C is produced from glutamate by a bifunctional P5C synthetase. The two pathways share the last reaction, in which P5C is reduced to proline by a P5C reductase. During the subsequent recovery from osmotic stress, proline is oxidized back to glutamate by the sequential action of Pro and P5C dehydrogenases. We found that the activity of both P5C-metabolizing enzymes of rice shows a complex pattern of post-translational regulation by salt and end-products. Moreover, in the case of P5C reductase inhibition or stimulation by salts was shown to depend on the use of NADH or NADPH as the electron donor. As a consequence, high intracellular ion concentrations are expected to immediately reduce proline catabolism in the mitochondrion, and to increase its synthesis in the cytosol, raising the overall proline content in the cell.This work was supported by AGER Foundation in the frame of the Risinnova project, grant # 2010-2369.

P19.4
Molecular networks controlling leaf cell differentiation during drought stress in *Brachypodium distachyon*

E. Bertolini[1], A. Mingrino[1], D. S. Horner[2], D. Inzè[3], M. E. Pè[1], E. Mica[1,4]
[1]Institute of Life Sciences, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa (Italy), [2]Department of BioSciences, Università degli Studi di Milano, Via Celoria 26, 20133 Milano (Italy), [3]Department of Plant Systems Biology, VIB, Technologiepark 927, 9052 Gent (Belgium), [4]Genomics Research Centre , Consiglio per la ricerca e la sperimentazione in agricoltura, Via San Protaso 302, 29017 Fiorenzuola d'Arda (Italy)

Plant response to drought is a complex trait involving well interconnected networks, leading to rapid reprogramming of plant growth., Here we present a comprehensive description of the molecular networks activated in response to drought, focusing on three different developmental zones (proliferation, expansion, and mature cells) of young *Brachypodium* leaf. To investigate the mechanisms controlling leaf growth reduction during drought, the third emerging leaf was dissected and subjected to whole transcriptome and small RNAs profiling based on next generation sequencing., SmallRNA-Seq data were analyzed using an in-house bioinformatics pipeline allowing the identification of 188 new miRNA genes, divided into known and novel families, and a large number of small interfering RNA-producing loci. mRNA-Seq data, produced on the same biological material, allowed us to better define the modulation of coding and non-coding genes, highlighting that distinct leaf zones respond differently to drought treatment. This approach provide novel evidence for a regulatory network where miRNAs and their targets genes are deeply interconnected, suggesting that drought alters cell cycle regulation.

P19.5
The PSI peripheral antenna is crucial for acclimation of *Arabidopsis thaliana* to changing light conditions

M. Bressan, S. Cazzaniga, R. Bassi, L. Dall'Osto
Laboratorio di Fotosintesi, Dipartimento di Biotecnologie, Università di Verona

PSI core of higher plants binds a peripheral antenna system (LHCI), composed by four light-harvesting proteins (Lhca1-4) and characterized by the presence of Chls absorbing at energy lower than the primary electron donor P700. The high conservation through evolution of both LHCI system and its spectroscopic peculiarities suggests a specific functional role. It has been hypothesized that LHCI increases the absorption of PSI in "shadelight" environment, or it is involved in chloroplast photoprotection, while its importance upon changes in light intensity has not been investigated. We have isolated an *A. thaliana* mutant devoid of the whole LHCI system*(ΔLhca)*. Lack of LHCI was not balanced by the over-accumulation of other LHCs. ΔLhca plants showed a reduced trans-thylakoid ΔpH gradient and limited growth. ΔLhca plants did not show a higher sensitivity to photoxidative stress when

exposed to excess light. Instead, in mutant plants both the redox balance between photosystems and the growth rate were strongly impaired under rapidly changing light intensities. We conclude that LHCI is crucial for the dynamic regulator of redox homeostasis in the thylakoid membrane of higher plants.

P19.6
Organ specificity of Strigolactone production in tomato plants under osmotic stress

I. Visentin[1], M. Vitali[1], C. Lovisolo[1], A. Schubert[1], F. Cardinale[1]
[1]Dept. of Agriculture, Forestry and Food Science (DISAFA), Turin Univ., Grugliasco (TO), Italy

Strigolactones (SLs) are a new class of plant hormones influencing various aspects of development and communication with soil (micro) flora, and proposed as mediators of environmental stimuli in resource allocation processes. Data collected in Arabidopsis and *Lotus japonicus* suggest that SLs in shoots, but not in roots, play a positive role in adaptive adjustments to drought. In this work, we tested the hypothesis that the biosynthesis of SLs, their effects on drought resistance and cross-talk with ABA are modulated oppositely in roots and shoots of tomato plants, both under normal and stress conditions. Data will be presented supporting the concept that during osmotic stress, SL synthesis is stopped in roots (where their effect would be negative on resistance, because they repress ABA synthesis) and transferred to leaves (where they are not produced under normal conditions, but where their presence promotes ABA sensitivity). We also hypothesize that under osmotic stress, the drop of the SL levels flowing upwards from roots to shoots may act as a long-distance, circuit breaker-signal conveying the root-generated stress signal to the stem.

P19.7
Enhanced production of bioactive compounds in plant cell suspension cultures of *Artemisia annua* L.

F. Rizzello, M. Durante, A. De Paolis, G. Mita, S. Caretto
CNR, Institute of Sciences of Food Production, Lecce Unit, Lecce, Italy

The herbal plant *Artemisia annua* L. is known to produce the antimalarial sesquiterpene lactone artemisinin (AN). We established *A. annua* L. cell suspension cultures able to produce intracellular and, more interestingly, extracellular AN. In a previous work, ß-cyclodextrins (ß-CDs) were demonstrated to highly increase AN in the culture medium of these suspension cultures. The aim of this work was to evaluate the effects of chemically modified β-CDs (2,6-dimethyl-β-cyclodextrins, DIMEB), at various time intervals, on the production of other bioactive isoprenoid compounds known as important antioxidants. Therefore, we analyzed both intracellular and extracellular levels of carotenoids and quinones in *A. annua* L. cell suspension cultures after treatment with DIMEB. The results showed a significant increase of carotenoids and quinones mostly in the spent medium of DIMEB-treated cells compared to the control, suggesting that DIMEB facilitate the accumulation of carotenoids and quinones in the culture medium, where they could be easily isolated. The influence of DIMEB on the expression of isoprenoid biosynthetic genes was investigated to assess the role of DIMEB as true elicitors.

P19.8
Molecular and technical advances for the *in vivo* analysis of Ca^{2+} dynamics in plant cells

MC. Bonza[1], G. Loro[1,2], A. Candeo[3], L. Fieramonti[3], S. Behera[1,4], L. Luoni[1], G. Valentini[3], F. Lo Schiavo[2], M. Zottini[2], J. Kudla[4], A. Bassi[3], A. Costa[1]
[1]Dipartimento di Bioscienze, Università degli Studi di Milano, [2]Dipartimento di Biologia, Università di Padova, [3]Departimento di Fisica, Politecnico di Milano, [4]Institut für Biologie und Biotechnologie der Pflanzen, Universität Münster,

In plants, rises in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) occur in response to both biotic and abiotic stimuli. These rises can display the form of a single transient or repetitive Ca^{2+} oscillations. Generation and shaping of $[Ca^{2+}]_{cyt}$ signatures depends on fine-tuning of Ca^{2+} influxes and effluxes occurring at both the plasma membrane (PM) and membranes of the different subcellular compartments., To understand how the cytosolic Ca^{2+} dynamics are generated and shaped two issues need to be addressed: i) the study of how do organelles participate in these processes and ii) the possibility to perform single cell Ca^{2+} analyses in complex tissues and organs, in natural context and in close-to-physiological conditions., To reach these objectives we are following complementary strategies: i) the *in planta* use of genetically encoded FRET-based Ca^{2+} Cameleon sensor for the analyses of Ca^{2+} dynamics in several subcellular compartments such as: cytosol, nucleus, mitochondria, peroxisomes and ER; ii) development of a new microscopy solution based on Light Sheet Microscopy and iii) Ca^{2+} imaging analyses in selected Arabidopsis knock out mutants (e.g. Ca^{2+}-ATPases and putative Ca^{2+} channels).

P19.9
Evaluation of new synthesized "adenosine derivatives" molecules as cytokinin-like compounds

F. D'Angiolillo[1], I. Giorgi[2], L. Spíchal[3], L. Pistelli[1]
[1]Dept Agriculture, Food and Environment, Pisa Univ., Pisa, Italy, [2]Dept Pharmacy, Pisa Univ., Pisa, Italy, [3]Laboratory of Growth Regulators, Palacký Univ. & Institute of Experimental Botany AS CR, Olomouc, Czech Republic

CKs are plant hormones that play regulatory roles in many aspects of plant growth and development as cell proliferation, shoot initiation, chloroplast biogenesis, inhibition of leaf senescence, and pathogen interactions. New synthesized "adenosine derivatives" molecules have structures related to that of cytokinins (CKs), commonly based on N6-substitued adenine structure. Classical CKs *in vivo* and*in vitro* assays (e.g. *Amaranthus caudatus* L. bioassay, cucumber cotyledon greening bioassay, and senescence bioassay) were carried out to evaluate the CK-activity of these "adenosine derivatives" molecules. Their hypothetical CK-like activity was compared with that of classical CKs, trans zeatin (tZ) and 6-benzilaminopurine (BAP). Positive results were obtained with ten molecules, further selected to check their ability to activate the CK signaling pathway leading to the biological response. The interaction with*At*CRE1/AHK4 and*At*AHK3 receptors was investigated, in order to evaluate the ability to bind CK-receptors; moreover the involvement signal transduction and/or to influence the CK signal cascade was examined with *ARR5*::GUS CK bioassay.

P19.10
The importance of osmoregulation and ionic effects on xylem hydraulics in the invasive halophyte *Spartina patens* (Ait.) Muhl

V. De Col[1], V. Casolo[1], M. Tomasella[2], E. Braidot[1], T. Savi[3], A. Nardini[3]
[1]Department of Agricultural and Environmental Sciences, University of Udine, [2]Department of Ecology, Technische Universität München, [3]Department of Life Sciences, University of Trieste

Spartina patens is a grass native of the Atlantic coasts of North America, which is now invading salt marshes in several coastal regions of Europe. In this work we investigate the leaf water relations and hydraulics, gas exchange, nitrogen and starch content in two populations of *S. patens* growing in a salt marsh and a dune system, aiming to understand the functional plasticity of the species. The analysis of leaf-level water relations revealed a number of mechanisms adopted by the species to overcome salt and drought stress, while leaf morphological traits and biomass accumulation were unaffected. In particular, marsh plants, exposed to more severe water stress, showed a larger osmoregulation and leaf hydraulic adjustment than dune plants. We also present the first experimental evidence for salt-mediated regulation of xylem hydraulic efficiency in a halophytic grass and we suggest that this might be a functional characteristic allowing this plant to cope with restricted water supply. The results suggest that functional plasticity of leaf water relations and xylem hydraulics might represent a key trait underlying the competitive ability and invasive potential of *S. patens*.

P19.11
The effects of heavy metals exposition on the activity of recombinant plant plastidic glucose 6P dehydrogenase

A. De Lillo, M. Cardi, M. Lentini, S. Esposito
Dept. Biology, Univ. Federico II of Napoli, Italy

Heavy metals (HMs) play an important role in plant metabolism: some are essential micronutrients, but their excess may become highly toxic to plants., HMs toxicity causes changes in cell structure and functionality, alterations of key metabolic processes, exchanges in enzymatic co-factors, and generation of free radicals. Furthermore, enzyme inhibition is always considered an important biomarker of response., Our study is focused on heavy metals effects on the activity of plastidic Glucose-6-phosphate dehydrogenase (G6PDH - EC 1.1.1.49), the key enzyme of the oxidative pentose phosphate pathway., This reaction provides the reductants for many metabolic processes in plant cells., These effects were analized by measuring the activity variations on recombinant P2-G6PDH isoform from *Populus trichocarpa* exposed to different levels of different heavy metals (Ni, Cd, Pb, Cu, Zn)., Ni and Cd caused a marked decrease in PtP2G6PDH activity, whereas Pb was almost uneffective. Interestingly, both copper and zinc exposition resulted in a strongest decrease in enzyme activity., These results are discussed in order to define a biochemical mechanism of inhibition of plant G6PDH by inorganic cations.

P19.12
Identification and molecular characterization of the sucrose synthase 2 gene in durum wheat

I. Fanizza[1], Lr. Ceci[2], G. Galante[2], B. Gattulli[2], C. Leoni[1], A. Gadaleta[3], D. Nigro[3], G. Mangini[3], A. Blanco[3], M. Volpicella[1]
[1]Department of Biosciences, Biotechnology and Biofarmaceutic , University of Bari "A. Moro", Via Amendola 162/A, 70126 Bari (Italy), [2]Institute of Biomembranes and Bioenergetic – CNR, Research Division Bari, [3]Department of Soil, Plant and Food Sciences Section Genetics and Plant Breeding, "A. Moro", Via G. Amendola 165/A - 70126 - Bari, Italy

Starch in the wheat endosperm is the major storage reserve of proteins, lipids and carbohydrates. The sucrose synthase (SUS) enzyme controls the flow of carbon into starch biosynthesis, catalyzing the first reaction in the conversion of sucrose to starch. The availability of genetic maps and phenotypic data of segregating population allows to map important genes, and to identify closely associated molecular markers to be used in MAS and positional cloning. The strategy adopted to identify the *Sus2* gene in the genomes of the durum wheat cvs. Ciccio and Svevo is showed. In order to obtain the complete sequence of the *Sus2* gene, a bioinformatic analysis starting from the cDNA sequence of *Sus2* gene in *Triticum aestivum* was performed. Specific primer combinations were designed and used in specific PCR reactions on the genomic DNA from leaf tissue of Ciccio and Svevo. The entire genomic structure of the *Sus2* genes were determined, and several SNPs, INDELs and variants were found in the comparison of the two homeologous alleles. A recombinant inbred lines developed from a cross the Svevo and Ciccio durum wheat cultivars will be used to genetic map the *Sus2* gene.

P19.13
Evolutionary role of a vacuolar metal transporter for hypertolerance/hyperaccumulation in *Arabidopsis halleri*

E. Fasani, G. DalCorso, A. Furini
Dept. Biotechnology, Univ. of Verona, Verona, Italy

VMT is a tonoplast metal transporter participating in vacuolar sequestration that is involved in metal hyperaccumulation/hypertolerance. The gene is constitutively expressed in metal hyperaccumulators, as *Arabidopsis halleri*, with higher transcript levels than in the corresponding non-accumulator species. In *A. halleri*, three different promoter sequences were identified for *VMT*, suggesting the presence of multiple gene copies, while a single copy is present in *Arabidopsis thaliana*. *VMT* promoter activity was compared in *A. thaliana* and *A. halleri* by GUS assay. All promoters are active in roots and guard cells, but *A. halleri* members drive GUS expression also in leaf mesophyll and trichomes. *In silico* analysis highlights, in the 5'UTR of the *A. halleri*promoters, a dimer of MYB-binding motifs, which is mutated in a single nucleotide in the *A. thaliana*sequence. Promoter mutation analysis indicates that this motif is likely involved in trichome-specific expression. The high *VMT* transcription levels observed in trichomes of *A. halleri*, counteracted by its absence in *A. thaliana* trichomes, suggest a putative evolutionary role of VMT in the hypertolerance/hyperaccumulation trait.

P19.14
Proline metabolism in salt-shocked *versus* salt-adapted rice seedlings

M. Bertazzini, R. Nansi Tchapnda, M. Zarattini, G. Forlani
Dept of Life Science and Biotechnology, University of Ferrara, Ferrara, Italy

Proline accumulation was investigated in rice (*Oryza sativa* L. cv Loto) seedlings grown in the presence of increasing concentrations of salt. A remarkable difference was found between salt-adapted and salt-shocked plants. In seedlings grown under normo-osmotic conditions proline levels rapidly raised following the treatment with NaCl. However, also the level of other free amino acids increased likewise, and the percentual content of proline varied only slightly. In contrast, under the same conditions tobacco seedlings showed a specific increase of free proline. On the contrary, in rice seedlings directly sown in the presence of inhibitory concentrations of salt the homeostatic level of most amino acids was maintained, with the only exception of proline and asparagine. In this case, proline percent values increased significantly. Results suggest that, contrary to other plant species, proline accumulation in rice is not a rapid mechanism for osmotic adjustment, but may represent a long-term adaptation to cope with the effects of excess salt in the environment. This work was supported by AGER Foundation in the frame of the Risinnova project, grant # 2010-2369.

P19.15
Peroxydase and polyphenoloxidase activities and isoforms distribution in fruits of sweet cherry from Campania Region (Italy)

A. Mirto , F. Iannuzzi, P. Woodrow, L. Ciarmiello, P. Carillo, A. Fuggi
Dept. Environmental, Biological and Pharmaceutical Sciences and Tecnologies, Second University of Naples, Caserta,Italy

Peroxidases and polyphenoloxidases play key roles in plant physiologic process, like lignin biosynthesis, responses to biotic and abiotic stresses, fruit ripening. Such enzymes are of main concern along the food supply chain, causing the deterioration of organoleptic properties of fruit and vegetable foods, Peroxidases and pholyphenoloxidases occurred in many isoforms, that have been used also as genetic markers in the identification of ecotypes and cultivars. In the aim of conservation and valorization of the agrobiodiversity, in this study peroxidases and polyphenoloxidases isoforms, have been characterized in fruits of sweet cherry landraces of Campania Region. The enzyme preparation were obtained from ripened cherry fruits harvested at commercial maturity. Soluble and bound peroxidases and polyphenoloxidases activities were measured in partially purified enzyme preparations. Peroxidase activities were about 10 times higher than polyphenoloxidase ones. They changed depending on the ecotype and on the phenolic substrate. Acidic, neutral and basic peroxidase isoforms were found, differently distributed among the ecotypes. Peroxidase enzymes from different landraces showed also different sensitivity to thermal denaturation Financial support was obtained by "Regione Campania (Italy), PSR 2007/2013, Misura 214, Azione f2, progetto Agrigenet".

P19.16
High-level expression of *Physcomitrella patens* light harvesting complex stress related protein 1 (LHCSR1) protein by transient expression in *Nicotiana benthamiana*

L. Ghin[1], A. Alboresi[2], A. Pinnola[1], M. Merlin[1], L. Avesani[1], R. Bassi[1]
[1]Dept of Biotechnology, University of Verona, Italy, [2]Dept of Biology, University of Padua, Italy

LHCSR is a protein involved in algae and mosses non-photochemical quencing (NPQ), the thermal dissipation of chlorophyll excited singlet states (1Chl*) necessary for the regulation of light use efficiency. LHCSR protein is present in a small amount in photosynthetic organisms and has so far escaped purification and characterization. The transient expression of *Physcomitrella patens LHCSR1* in *Nicotiana benthamiana* was performed. *LHCSR1* gene and *LHCSR1* gene carrying a His Tag sequence at C-terminus were cloned in two different plasmids: pK7WG2, a gateway destination vector, and pPXVX201, a plasmid containing the cDNA encoding the complete Potato virus X. *Agrobacterium tumefaciens* mediated leaves infection was used for plants transformation. Accumulation of mature proteins was confirmed by Western Blot analysis, showing a comparable level of expression between the two different plasmids. Purification protocols are being optimized to characterize and localize LHCSR1 and to define its mechanism of action.

P19.17
Metal adaptation can affect the response to biotic stress in higher plants: a study on *Silene paradoxa* L.

C. Taiti[1], S. Luti[2], N. Bazihizina[1], I. Colzi[3], E. Giorni[3], S. Mancuso[1], L. Pazzagli[2], C. Gonnelli[3]
[1]Dip. Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università di Firenze, Italia., [2] Dip. Scienze Biomediche Sperimentali e Cliniche, Università di Firenze, Italia., [3] Dip. Biologia, Università di Firenze, Italia

Ceratoplatanin (CP), a protein with PAMP activity (1), was found to induce, in presence of Cu, a higher production of phytoalexins in a Cu tolerant population of Silene paradoxa L in respect to a sensitive one (2). Here we evaluated CP induced volatile organic compounds (VOCs) emitted by S. paradoxa in presence/absence of Cu, as biotic stress can trigger VOCs emission for the activation of defense pathways (3). VOCs were detected by PTR-MS after 1,4 and 24 h from spraying a 3mM CP solution on plants. Tolerant population produced larger amount of VOCs than sensitive population and especially in presence of 5μM CuSO4 in the medium. After 1 h compounds with m/z of 63.027, 71.049 and 101.096 m/z were measured and tentatively identified as dimethylsulfide, vinyl ketone and hexanal, respectively. After 24h Cu tolerant plants showed a higher production of terpens and sesquiterpens compared to sensitive plants. Results confirmed that in presence of Cu tolerant polulation produced more defence responses, upon CP treatment, when compared to sensitive population., 1)Plant Sci doi: 10.1016/j.plantsci.2014.02.009, 2)Env Exp Bot doi: 10.1016/j.envexpbot.2013.11.014, 3)Front Plant Sci 2013, 4, 262

P19.18
Leaf water relation traits in typical Sicilian varieties of *Vitis vinifera* L.

S. Inzerillo[1], E. Oddo[1], L. Pollina[1], L. Abbate[2], F. Carimi[2], M. Sajeva[1], A. Nardini[3]
[1]Dept STEBICEF, University of Palermo, via Archirafi 20, Palermo, Italy, [2]Institute of Biosciences and BioResources-CNR, Corso Calatafimi 414, Palermo, Italy, [3]Dept Scienze della Vita, University of Trieste, via Giorgieri 10, Trieste, Italy

In Italy, grapevines are extensively cultivated, with Sicily representing one of the most significant wine regions. The high number of autochthonous grapevine varieties represents an important source of genetic diversity, and the many Sicilian varieties have anatomical and physiological traits that allow them to resist to different levels of drought stress. We investigated the water relation parameters of four cultivars of *Vitis vinifera*L. (Catarratto, Corinto, Nero d'Avola and Zibibbo) and characterized their leaf hydraulics. Measurements were conducted during summer on plants growing in the experimental field of the IBBR-CNR near Palermo. Daily patterns of leaf water potential (Ψ_{leaf}) and stomatal conductance (g_s) were measured in the field. Pressure-volume curves were constructed by the bench dehydration method to obtain leaf water potential at turgor loss point (Ψ_{tlp}), osmotic potential at full rehydration (π_0) and bulk modulus of elasticity (ε_{max}). Leaf samples were collected to determine vein density using ImageJ. Major vein density was measured on digitally scanned leaves, while minor vein density was measured on photomicrographs of cleared and stained leaf portions.

P19.19
Phenotypic plasticity of Mediterranean oak species in response to combined ozone and drought stress

M. Landi, L. Cotrozzi, E. Pellegrini
Department of Agriculture, Food & Environment, University of Pisa, Pisa, Italy

Three Mediterranean oak species (*Quercus ilex*,*Q. pubescens* and*Q. cerris*) were selected to assess the plasticity of biometrical, biochemical and physiological traits under drought (daily irrigation: 30% of effective evapotranspiration) and O_3 [90 ppb (5 h daily) for 77 days] stress. At the end of the experiment, despite all treated plants showed marked phylloptosis, only drought or drought x O_3 treatment caused minute necrosis over the leaves. 0 to 1 index of phenotypic plasticity (PI) was calculated for each parameter and species. All species were characterized by comparable values of plasticity for morpho-anatomical traits, while a higher level of physiological plasticity was found for two species selected for their drought tolerance, *Q. ilex* (PI=0.49) and*Q. pubescens* (PI=0.54) *vs Q. cerris* (PI=0.36). Of note, a higher plasticity of biochemical traits found in *Q. ilex* (PI=0.37 *vs* 0.27 and 0.24 in *Q. pubescens* and *Q. cerris*, respectively) resulted meanly attributable to a high ability to modulate proline content under stress conditions. The plasticity of that mechanism likely significantly contributes to the higher tolerance against drought and O_3demonstrated by *Q.ilex*.

P19.20
Changes in the levels of HSP70 in relation to heavy metals exposition in *Elodea canadensis*

M. Lentini, M. Cardi, A. De Lillo, A. Basile, S. Esposito
Dept Biology – Univ. of Napoli Federico II, Italy

Heavy metal pollution represents one of the worst pollution in Earth ecosystems., The description of suitable biological plant indicators, and appropriate biomarkers witnessing the heavy metals stress has been one of the scientific tasks in the last decades., In this study we utilised Elodea canadensis (Hydrocharitaceae) as a bio-indicator, and Hsp70 occurrence as a protein biomarker to evaluate the effects of different heavy metals., In details, the occurrence and abundance of Hsp70 protein were investigated by changing both levels and time of exposure using different heavy metals on *in vitro* cultured specimens., We used Hsp70 antibodies able to recognize cytosolic and chloroplastic isoforms, in order to recognize which of them is/are specifically involved in response to heavy metals stress, by western blotting., The possible relationship(s) between the presence and the abundance of different isoforms, to changing heavy metals stress exposition and concentrations, are discussed in order to define this method as a useful tools to rapidly assess and monitor heavy metals pollution in plants.

P19.21
Interplay between cGMP and redox signalling under biotic stress

P. Fincato[1], E. Vandelle[2], F. Orsini[1], D. Bellin[2], M. Delledonne[2], L. De Gara[1], V. Locato[1]
[1]Lab. Plant Biochemistry and Food Sciences, Università Campus Bio-

Medico, Rome, Italy, [2] Dep. Biotechnology, Università degli Studi di Verona, Verona, Italy

The cyclic nucleotide cGMP is a key second messenger involved in plant developmental processes as well as in stress defense. Its mechanism of action in plant is still poorly characterized. It has been suggested that cGMP is involved in the regulation of the oxidative burst induced during the HR by avirulent pathogens. This oxidative process also requires a tight regulation of the ROS scavenging systems. In particular ascorbate-glutathione cycle has been reported to be strongly altered as part of the programmed cell death occurring during HR., In order to identify possible cross-talks between cyclic nucleotide-dependent and redox-mediated signaling pathways, a biochemical and molecular characterization of transgenic *Arabidopsis thaliana* plants displaying a high cGMP content has been performed in both control conditions and following attack with an avirulent strain of *Pseudomonas syringae.* In particular the alterations of metabolites and enzymes involved in redox homeostasis as well as redox dependent post-translational modifications have been analyzed during the time course of infection.

P19.22
Aquaporin involvement in controlling leaf hydraulic capacitance and resistance

M. Vitali[1], H. Cochard[2], A. Ponomarenko[2], A. Schubert[1], I. Perrone[1,3], G. Gambino[3], C. Lovisolo[1]
[1]University of Turin, Dept Agricultural, Forest and Food Sciences, Grugliasco, Turin, Italy, [2] UMR-PIAF, INRA-Université Blaise Pascal, Clermont-Ferrand, France, [3] CNR, Institute for Sustainable Plant Protection, Grugliasco Unit, Turin, Italy

Hydraulic capacitance (Ch) and resistance (Rh) were assessed on detached leaves sampled from grapevines overexpressing the aquaporin gene *VvPIP2;4N* by following recovery dynamics after leaf dehydration. The dehydration was imposed by submitting the leaves at 0.5 to 1 MPa overpressure through a Scholander bomb. The recovery after dehydration was checked in dark, in light non-transpirative condition, and in light-transpirative condition. In leaves processed in dark conditions, Rh decreased and Ch increased compared to wild type plants, suggesting that in transgenic leaves, trans-cellular pathways activated during dehydration superimposed on apoplastic ones, saving water inside cells, as in/out cell-to-apoplasm water movements were aquaporin-controlled. Upon light, either when leaves transpired or not (either by depressing vapor pressure deficit or by feeding leaves with abscisic acid, causing stomatal closure) the described effects disappeared. We conclude that light activates leaf aquaporins masking the transgene effect on controlling leaf Ch and Rh, and confirming that the in-vivo leaf hydraulics is not affected by transgenic aquaporin *VvPIP2;4N* overexpression.

P19.23
Rooting depth as a key trait connecting water and carbon metabolism of trees

A. Dal Borgo[1], A. Nardini[1], V. Casolo[2], T. Savi[1], A. Bressan[2], B. Stenni[3], L. Zini[3]
[1]Dept Life Sciences, Univ. Trieste, Italy, [2] Dept Agricultural & Environmental Sciences, Univ. Udine, Italy, [3] Dept Mathematics & Geosciences, Univ. Trieste, Italy

Drought and heat waves are thought to be the main cause of increasing rates of tree die-back in several biomes. Three possible and not mutually exclusive mechanisms have been proposed to be the drivers of this phenomenon: hydraulic failure caused by massive xylem cavitation and leading to plant desiccation, carbon starvation caused by prolonged stomatal closure and leading to impairment of primary and secondary metabolism, and finally biotic attacks. The different mechanisms have been reported to have different relevance in the different species. On the basis of the analysis of seasonal changes of water relations, xylem sap isotopic composition, and concentration of non-structural carbohydrates in different woody species during a summer drought, we propose that rooting depth is a key trait connecting water and carbon plant metabolism, thus mediating the likelihood of hydraulic failure vs carbon starvation in drought-stressed trees.

P19.24
Potassium deficiency and drought stress in grapevine cultivars

E. Oddo[1], L. Abbate[2], S. Inzerillo[1], M. Calabrese[1], F. Carimi[2], A. Motisi[2], M. Sajeva[1], A. Nardini[3]
[1]Dept STEBICEF, University of Palermo, via Archirafi 20, Palermo, Italy, [2] Institute of Biosciences and BioResources-CNR, Corso Calatafimi 414, Palermo, Italy, [3] Dept Scienze della Vita, University of Trieste, via Giorgieri 10, Trieste, Italy

Potassium availability affects drought responses in plants through several metabolic roles, among which stomatal regulation, cell growth and xylem hydraulics. *Vitis vinifera* L. is a highly valuable crop and several genotypes have been selected during its millennial cultivation. Varieties show differences in their adaptability to stress conditions, making them more or less suitable to certain climatic and edaphic conditions. The varieties cultivated in Sicily are characterized by high variability. We investigated the response of two Sicilian cultivars (Nero d'Avola and Catarratto) to potassium deficiency and drought stress. Two-year-old grafted plants were grown in agriperlite, with or without potassium in the fertigation solution, and subjected to moderate drought stress by suspending irrigation for 6-8 days. Potassium content of xylem sap, leaf and root tissues were measured with an ion-selective electrode. Changes in stomatal conductance, plant transpiration and hydraulic conductance were compared between genotypes and treatments, in order to gain information for the development of optimal fertigation practices and the selection of the most drought tolerant varieties.

P19.25
Defense response to fungal pathogens in susceptible and resistant maize genotypes

C. Paciolla, M.P. Bancone, I. Giannella, S. De Leonardis
Dept Biology, "Aldo Moro" Bari Univ., Bari, Italy

Pathogen interactions can lead to the increased production and/or accumulation of ROS in plant tissue. To reduce and scavenge ROS, the plant have enzymatic and non-enzymatic defense systems. The aim of present work is to analyze in maize kernels of susceptible (CO354) and resistant (CO441) genotypes, at 15 days after anthesis, the response to inoculation with necrotrophic (*Aspergillus flavus*) and hemibiotrophic pathogens (*Fusarium proliferatum*and*F. subglutinans*) that cause ear rot. After 72 hours, in uninoculated and inoculated kernels, the ascorbate-glutathione (ASC-GSH) cycle, superoxide dismutase, peroxidase and catalase, playing an important role in the cell detoxification by ROS, were analyzed. All components of ASC-GSH cycle and the ROS scavenging enzymes already in the uninoculated resistant controls were present at higher level. In the susceptible line an increase in glutathione reduced, ascorbate, H_2O_2, and malondialdehyde content was observed post-inoculation, suggesting that metabolic perturbations in these kernels caused alteration of cell redox status and enhanced oxidation of the cytosol. CO441 resistant genotype could be interesting line to be utilized in breeding programs focused on the resistance to fungal pathogens of maize kernel., This work was supported by the PRIN (20094CEKT4) of the MIUR, Italy.,

P19.26
Novel putative miRNAs in different graft combinations of grapevine upon drought

C. Pagliarani, M. Ferrero, M. Vitali, C. Lovisolo, D. Alberto, A. Schubert
Dept Agricultural, Forest and Food Sciences, Univ. of Turin, Turin, Italy

We investigated the expression changes induced by drought on a group of novel putative miRNAs and related target transcripts in leaves and roots of *Vitis vinifera* cv. Cabernet Sauvignon (CS) and M4 rootstock (*V. vinifera* X *V. berlandieri*), and of their auto- and reciprocal grafts., A 10-day drought treatment was imposed onto target grapevines: gas exchanges were daily monitored on treated (WS) and irrigated (IRR) plants until the moment of maximum stress chosen for sampling (g_s < 0.05 mmol H_2O

m^{-2} s^{-1} and Ψ_{leaf} ~ -1.4 MPa). RNA samples from WS and IRR leaves and roots of the two genotypes (CS, M4) and of different graft combinations (CS/CS, M4/M4, CS/M4, M4/CS) were processed to carry out both high-throughput sequencing and RT-qPCR analyses. Sequencing data allowed to identify 13 novel miRNAs, whose expression profiles were further analyzed by RT-qPCR together with those of their target transcripts, previously predicted *in silico*., The results show that the abundance of the analyzed novel miRNAs is affected by the genotype, tissue and graft combination, suggesting miRNA transport events between rootstock and scion., Acknowledgments: AGER-SERRES N° 2010-2105 and Fondazione CRC–INTEFLAVI.

P19.27
Metabolite transport pathways in durum wheat mitochondria from hyperosmotically-stressed seedlings

D. Trono[1], M.N. Laus[2], M. Soccio[2], D. Pastore[2]
[1]CRA-CER, Foggia, Italy, [2]Dept. SAFE, Univ. of Foggia, Foggia, Italy

In durum wheat mitochondria (DWM) the ATP-inhibited plant mitochondrial potassium channel ($PmitoK_{ATP}$) and the plant uncoupling protein (PUCP) are able to strongly reduce the proton motive force (pmf) to control mitochondrial production of reactive oxygen species (ROS), which is known to increase under environmental stresses. In particular, in hyperosmotically stressed DWM, both $PmitoK_{ATP}$ and PUCP are activated by the increased ROS production and by an increase, observed under this adverse condition, in FFAs released by a stress-induced mitochondrial PLA_2, and probably by their acyl-CoA derivatives. Under this condition, mitochondrial carriers lack the driving force for transport and should be inactive; however, unexpectedly, DWM uncoupling by $PmitoK_{ATP}$ neither impairs the ADP/ATP exchange nor blocks the Pi and succinate transports. This uptake may occur via the plant inner membrane anion channel (PIMAC), which is physiologically inhibited by membrane potential, but unlocks its activity in de-energized mitochondria. Probably, cooperation between PIMAC and classical carriers may accomplish metabolite movement across the inner membrane under both energized and de-energized conditions.

P19.28
Redox status of the pyridine nucleotide pools and proline synthesis in tobacco

D. Petrollino , R. Nansi Tchapnda, M. Bertazzini, G. Forlani
Dept of Life Science and Biotechnology, University of Ferrara, Ferrara, Italy

Proline is the best-studied compatible osmolyte that accumulates in plants in response to water deficit, yet the full range of protective functions are still to be revealed. Even a small increase in the rate of proline synthesis during stress is believed to have a large impact on the level of reduction of the intracellular $NADP^+$ pool. An increased $NADP^+$/NADPH ratio can promote the activity of the oxidative pentose phosphate pathway, providing in turn energy and precursors to support the demand for increased secondary metabolite production. Consistent properties were recently found in Arabidopsis for the enzyme that catalyzes the second and last step in proline synthesis, P5C reductase. At physiological levels, $NADP^+$ almost suppressed the NADH-dependent activity, and increasing concentrations of salt inhibited or stimulated P5C reductase depending on the use of NADH or NADPH as the electron donor. Based on these results, however, also the opposite may be true, *i.e.* variations in the redox status of the pyridine nucleotide pools could affect the rate of proline synthesis. Here we report preliminary data linking $NAD(P)^+$/NAD(P)H ratio and proline homeostasis in tobacco seedlings.

P19.29
Redox regulated β-amylase 1 (BAM1) is inhibited by glutathionylation

C. Pirone, G.L. Borghi, P. Trost, F. Sparla
Dept of Pharmacy and Biotechnology, Univ. of Bologna, Bologna, Italy

The chloroplastic β-amylase 1 (BAM1) is the only thioredoxin-regulated β-amylase of Arabidopsis. BAM1 activity is inhibited via disulfide bridge formation and activated by reductants. *In vivo*, BAM1 plays a role in diurnal starch degradation in guard cells, and is involved in osmotic stress responses in mesophyll cells (Valerio et al., 2011). The possibility that BAM1 may also be affected by other types of post translational modification (PTMs) that occur under oxidative stress was investigated. BAM1 activity was assayed with the artificial substrate PNPG3 and found to be inhibited by different oxidative treatments (e.g. GSSG, H2O2, H2O2 plus GSH). Interestingly, the activity of GSSG-treated BAM1 was fully reverted by chemical reduction with DTT, and BAM1 was demonstrated to be glutathionylated by biotin-labeled GSSG, suggesting a role of glutathionylation in protecting BAM1 from irreversible oxidation under oxidative stress conditions.

P19.30
Phytoremediation potentiality of *Cistus ssp.* and *Inula viscosa* in heavy metal contaminated soils

L. Pistelli[1], F. D'Angiolillo[1], E. Morelli[2], M. Barbafieri[3], B. Basso[2]
[1]Dipartimento di Scienze agrarie, alimentari e agro-ambientali, Università di Pisa, [2] Istituto di Biofisica del CNR (Pisa), [3]Istituto per lo Studio degli Ecosistemi del CNR (Pisa)

Aim of the study is the evaluation of native Mediterranean plants, e.g. *Cistus* ssp and*Inula viscosa*, to be used for the phytoremediation of dismissed mining areas on Elba Island. The study was focused on the area named Puppaio in the Mining Park near Rio Marina. Different parameters were considered: a) heavy metal composition of the soil; b) collection and sampling of *Inula viscosa* (L.) Aiton, *C. monspeliensis L.*and*C. salvifolius* L. growing on contaminated soil; c) chemical analysis of different plant organs (roots, stems, leaves) in order to evaluate metal uptake and translocation; d) activity of the main antioxidant enzymes and presence of secondary metabolites linked to oxidative stress; e) *in vitro* cultures of the selected species, grown on specific media mimicking some of the original conditions (presence of heavy metals, extremely acidic pH), in order to obtain a population of plantlets able to be possibly reintroduced in the considered polluted areas. Preliminary results showed: a) the ability of collected plants to take up and translocate metals to the aerial parts, b) an enhanced ability of the native plants to grow in media at low pH and high metal concentrations.

P19.31
Redox regulation of algal ATP sulfurylase

L. Prioretti[1], R. Lebrun[2], B. Gontero[3], M. Giordano[1]
[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy, [2]Plateforme Protéomique, FR3479, IBiSA Marseille-Protéomique, CNRS, 31 Chemin Joseph Aiguier, BP71, 13402 Marseille Cedex 20, France, [3]Aix-Marseille Université CNRS, BIP UMR 7281, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France

ATP sulfurylase (ATPS) is an enzyme of primary importance for photosynthetic organisms since it catalyzes the first reaction of the sulfate assimilation pathway., ATPS was highly studied in plants and fungi, but little is known about the algal enzyme and no attempts have been done yet to study its possible redox regulation., We used a bioinformatic approach to show that ATPS from eukaryotic algae and marine cyanobacteria possesses a much higher cysteine content than ATPS from other organisms. The activity of this enzyme is stimulated by treatment with reducing agents but inhibited by oxidizing agents. In contrast, the activity of ATPS from freshwater and less representative marine cyanobacteria is stimulated either by reducing and oxidizing conditions., Since this redox regulation is connected to the amount and conservation of the cysteine residues, we purified for the first time the ATPS from the marine diatom *Thalassiosira pseudonana*and the freshwater cyanobacterium *Synechocystis*sp., Therefore we evidenced that the sulfhydryl-reactive reagent iodoacetic acid inhibited the reduced enzyme completely, indicating that cysteine residues are critical amino acids for the activity of ATPS.

P19.32
Biochar amendment improves lettuce quality in metal-contaminated soils

M.F. Quartacci, C. Sgherri, A. Ranieri
Dept Agriculture, Food and Environment, Pisa Univ., Pisa, Italy

Biochar produced by the combustion of fresh plant biomass is able to improve the physic-chemical characteristics of soils. The possibility to use biochar, which also adsorbs toxic compounds preventing or limiting their entry in the food chain, may give both strategic and economic advantages., The research has taken into consideration some aspects related to food safety and in particular the exclusion from food of toxic substances present in the soil. Indeed, the accumulation of these compounds in plants, besides negatively influencing crop yields, causes an alteration of food quality and the reduction or disappearance of healthy nutraceutical molecules considered to be preventive agents of the most common diseases., The study aimed to i) evaluate the effect of biochar amendment on the uptake of copper by lettuce, and ii) to study whether copper concentration influenced the antioxidative power of lettuce as well as the concentration of some protective compounds (ascorbate, glutathione, phenolics) important for human health.

P19.33
Flax seed and its by-product as a source of secondary metabolites

A. Pugliese, D. Cola, A. Castagna, A. Ranieri
Dept. of Agriculture, Food and Environment

The possibility to integrate the human nutrition with products resulting from flax processing, is a promising way to increase the supply of nutraceutical compounds. It is known that flax represents an excellent source of antioxidants mainly due to the presence of carotenoids and phenolic compounds.The purpose of this study was to verify whether in the oilcake obtained by cold pressing of flax seeds were maintained the healthful features typical of the seed. The influence of the length of the storage and the type of packaging (paper or plastic) in determining the content of the major nutraceuticals, such as carotenoids, chlorophylls, phenolics, together with the assessment of their antioxidant capacity, were evaluated by different techniques.The results showed the same composition of bioactive compounds, as well as for the antioxidant activity, between seed and oilcake. A limited decrease in the parameters considered occurred after 6 months of storage of the oilcake, regardless the mode of packaging.In conclusion, the results suggest that the co-products of the agri-food industry, currently considered as waste, may be used as a good resource for human and animal nutrition.

P19.34
Xylem cavitation and hydraulic degradation portend increasing risks of tree mortality under climate change scenarios

T. Savi, M. Novak, J. Luglio, A. Nardini
Dept Life Sciences, Univ. Trieste, Italy

Extreme drought events accompanied by heat waves are posing a threat to productivity and even survival of trees in several forest biomes., Drought-induced tree die-back is thought to be triggered by hydraulic failure caused by xylem embolism. In turn, stomatal closure to prevent or in response to massive xylem cavitation can lead to carbon starvation, eventually favoring post-drought biotic attacks or impairing mechanisms underlying hydraulic recovery. We present data recently obtained on *Fraxinus ornus* L. and Pinus nigra J. F. Arnold suggesting that extreme drought events imply an hydraulic legacy in terms of increased vulnerability to cavitation and/or decreased hydraulic efficiency, possibly leading to long-term reduction of photosynthetic rates. Our, data also reveal possible functional differences between Angiosperms and Gymnosperms in terms of responses to extreme drought events.

P19.35
UV-B post-harvest irradiation is effective in increasing the level of antioxidants of peaches during a brief refrigerated storage

C. Scattino[1], A. Castagna[1], P. Tonutti[2], A. Ranieri[1]
[1]Dept of Agriculture, Food and Environment, University of Pisa, [2]Institute of Life Science, Scuola Superiore Sant'Anna, Pisa

Previous researches conducted on peaches cv 'Suncrest' showed that a post-harvest UV-B irradiation (1.68 W/m^2) was effective in ameliorating quality attributes and the concentration of antioxidants. The aim of the present study was to verify whether the eliciting effects triggered by UV-B exposure on the same cultivar were transient or, conversely, maintained during a brief storage in refrigerated condition. Peaches were UV-B treated for 24 h at 20°C. Then, fruits were stored up to 36 h at 10°C. The concentrations of the reduced form of ascorbic acid was positively affected by UV-B, as well as the ascorbate redox state after 36 h. The concentration of phenolic compounds, measured by different techniques, was affected in a positive way by 24 h irradiation and this tendency continues in peaches stored for 36 h. These results indicate that UV-B is able to trigger a series of metabolic changes lasting after the removal of the eliciting factor. Accordingly, UV-B deserves further investigations as tool capable of improving the contents of health-promoting compounds in peach fruits, as well as to increase the antioxidant contents of sub-optimal fruits or after long–term storage.

P19.36
The response of *Arabidopsis thaliana* to mild osmotic stress involves primary starch metabolism and is triggered by the activation of the redox sensitive ß-amylase 1 (BAM1)

M. Zanella[1,2], G.L. Borghi[1], D. Pazmino[2], C. Pirone[1], A. Costa[3], P. Trost[1], D. Santelia[2], F. Sparla[1]
[1]Dept Pharmacy and Biotechnology, Univ. of Bologna, Italy, [2]Inst Plant Biology, Univ. of Zürich, Switzerland, [3]Dept Bioscience, Univ. of Milan, Italy

Through photosynthesis, plants absorb light energy to convert CO_2 into glucose. Glucose is used for respiration, converted into sucrose for export or converted into starch for storage. In leaves, primary starch is synthesized during the day and stored in the chloroplast, where it is degraded during the subsequent night to fuel plants when the photosynthetic processes cannot occur., Beta-maltose, exclusively produced by ß-amylases, is the first soluble product of primary starch degradation. Among the different ß-amylase isoforms of Arabidopsis (nine in total), BAM3 is responsible for nocturnal starch degradation occurring under physiological conditions (Kötting et al., 2010), while BAM1 is redox-sensitive and involved in diurnal starch degradation in response to abiotic stress (Valerio et al, 2010)., By comparing the responses of wild type plants with *bam1* and *bam3* T-DNA insertional mutants, here we show that mild osmotic stress induces *BAM1* expression and triggers diurnal starch degradation, apparently to sustain sucrose and proline biosynthesis for protection against oxidative damage.

P19.37
iTRAQ analysis of the molecular dialogue between an arbuscolar mycorrhizal fungus and its endosymbiotic bacteria

L. Testa[1], C. Vannini[1], A. Carpentieri[2], A. Amoresano[2], A. Salvioli[3], M. Novero[3], M. Bracale[1], P. Bonfante[3]
[1]University of Insubria, Varese, Italy, [2]Università di Napoli Federico II, Napoli, Italy, [3]University of Torino, Torino, Italy

The endobacteria *Candidatus Glomeribacter gigasporarum* confer an ecologically relevant fitness to their host, the arbuscolar mycorrhizal (AM) fungus *Gigaspora margarita*.The endobacteria live in the cytoplasm of fungi and respond also to signals of plant origin, as strigolactones (SLs).In response to a SL treatment, the number of the dividing bacteria increases and influences hyphal elongation and branching.

The interaction pathways between AM fungi and their endobacteria are still not well understood.In this work, LC-MS/MS quantitative proteomic based approach was used for gaining new insights into the complex cellular crosstalk accompanying this symbiosis.We compared germinating fungal spores in the presence (wt) and the absence (cured) of the bacterial components and we tested the effects of treatments with a strigolactone analogue.In order to identify the differentially expressed proteins in four different samples, aliquots were labeled with iTRAQ reagents and analyzed by nano-LC-MS/MS. Samples were identified by Mascot software and protein expression evaluated by Scaffold software. Qualitative and quantitative differences both in fungi and in bacteria proteins will be presented.

P19.38

Photosynthetic response to nitrogen starvation and high light in *Haematococcus pluvialis*

L. Scibilia[1], A. Alboresi[1,2], M. Ballottari[1]

[1]Department of Biotechnology, University of Verona, Strada Le Grazie, I-37134 Verona, Italy, [2]Present address: Department of Biology, University of Padova, via U. Bassi 58/B, 35121 Padova, Italy

The carotenoid astaxanthin is mainly produced by microalgae and in particular by the freshwater green alga *Haematococcus pluvialis*. Its production is stimulated by exposing *H. pluvialis* cells to stressing conditions as high irradiances and nitrogen starvation. In this work high light and nitrogen deprivation stresses were applied to study their influence on the photosynthetic properties, chlorophylls and carotenoids biosynthesis, growth rates, oxygen consumption/evolution and PTOX activity in *H. pluvialis*. Our results demonstrate that photosynthetic properties of *H. pluvialis*are differentially modulated in response to various stress conditions:nitrogen starvation inhibits chlorophyll biosynthesis, promotes chlorophyll b degradation, PTOX activity and favors respiration over photosynthesis, while high light mainly activates xanthophyll cycle and carotenogenesis. The combined exposure of *H. pluvialis* to high light and nitrogen starvation strongly induce a significant reduction of functional PSII antenna size and increase astaxanthin production compared to nitrogen starvation or high light only stresses, improving the resistance of cells to photo-oxidation.

P19.39

Does F_OF_1 ATP synthase form the mitochondrial permeability transition pore in plants?

S. Patui[1], C. Peresson[1], V. De Col[1], E. Braidot[1], E. Petrussa[1], V. Casolo[1], A. Bertolini[1], G. Lippe[2], A. Vianello[1], M. Zancani[1]

[1]Dept of Agricultural and Environmental Science, Plant Biology Unit, University of Udine, Italy, [2]Dept of Food Sciences, University of Udine, Italy

Recently, it has been proposed that in mammals the mitochondrial permeability transition pore (PTP) is formed by F_OF_1 ATP synthase dimers. The permeability transition in plant mitochondria is still elusive and has been evidenced only in few peculiar cases. This research aimed at comparing the components and activity of plant mitochondria ATP synthase to its mammalian counterpart. Purified mitochondria were subjected to BN-PAGE, and the gel was stained for ATPase activity. The active bands, after immunodecoration with specific primary antibodies, confirmed the presence of the PTP inducer CyPD, associated to ATP synthase. ATP synthase was also characterized in sub-mitochondrial particles, evaluating its activity as both ATP hydrolysis and proton pumping. The scalar ATPase activity was dependent on divalent cations, while ATP induced the formation of a proton gradient only in the presence of Mg^{2+} and Mn^{2+}, being Ca^{2+} ineffective. Our results show that ATP synthase in plant mitochondria possesses similar structural and functional properties with respect to the animal counterpart, suggesting it might switch to PTP under stress conditions. *Supported by MIUR grant PRIN 2010CSJX4F.*

P19.40

Rare earth elements: resources or pollutants?

M.A. Zicari[1], A. Paradiso[1], L. d'Aquino[2], F. Tommasi[1]

[1]Dept Biology, Aldo Moro Univ., Bari, Italy, [2]ENEA, Research Center, Portici, Italy

The Lanthanides, or "rare earth elements" (REEs), are a group of 15 elements from lanthanum to lutetium including yttrium and scandium, with similar chemical and toxicological properties. Increasing utilization of REEs for many high technology applications and in agriculture aimed to enhance crop production increased the concern about a possible accumulation of such elements in the ecosystem. Information about toxicity in plants and animals are still contradictory. Cerium (Ce) and lanthanum are the most abundant REEs in the environment and their similarities to calcium (Ca) enhances absorption of such elements in plant tissues where those can displace and compete with Ca in Ca-mediated biological processes, possibly with negative effects on plant physiology. The toxicity of Ce to plants can be greater than that induced by other REEs because of its unique ability to change oxidation states. The biological risk associated with spreading of Ce in the environment is still poorly known and monitoring its biological effects will, therefore, be necessary. The effects of Ce on the growth and antioxidant metabolism were studied in *Lemna minor*, a test species for ecotoxicological assays.

O19.1

Proteome of *Triticum aestivum* cv Bologna affected by free – air CO_2 enrichment

F. Verrillo[1], L. Testa[1], V. Terzi[2], F. Rizza[2], F. Badeck[2], M. Bracale[1], C. Vannini[1]

[1]Departments of Biotecnology and Life Science (DSBV), University of Insubria, Via J. Dunant, 3, 21100 Varese, [2]CRA-GPG, Centro di Ricerca per la Genomica e la Postgenomica Animale e Vegetale, Via San Protaso 302, 29017-Fiorenzuola d'Arda (PC)

The increasing concentration of atmospheric CO_2 and the nutritional quality of human diets are the two important issues we are facing. At present,the atmospheric CO_2 concentration is about 380 ppm,but it is expected to reach 550 ppm by 2050.Elevated atmospheric CO_2 concentration is predicted to affect plant growth,yield and leaf photosynthesis. In order to obtain information about CO_2 effects on wheat growth and grain quality and composition,we analyzed for two years the behavior of *Triticum aestivum* cv Bologna in free–air CO_2 enrichment (FACE) field experiments which allow the exposure of plants to elevated CO_2under natural and fully open-air conditions. CO_2 enrichment (570 ppm) promoted wheat aboveground biomass and carotenoids accumulation.However,crude protein content and harvest index declined under FACE conditions.Comparative proteomic approach based on 2DE was adopted to identify proteins altered by FACE treatment.Differentially expressed proteins were determined in the sample by means of proteome maps statistical analysis ($p<0.05$) and further identified by LC- ESI-MS approach.Our experiments demonstrates that high CO_2 affect significantly the proteome of mature wheat grain.

O19.2

Involvement of DNA methylation in the control of cell growth during heat stress

I. Centomani[1], A. Sgobba[1], N. Dipierro[1], A. Paradiso[1], L. De Gara[2], S. Dipierro[1], L. Viggiano[1], M.C. de Pinto[1]

[1]Dept Biology, "Aldo Moro" Bari Univ., Bari, Italy, [2]CIR, Campus Bio-Medico Univ., Rome, Italy

Under adverse environmental conditions plants are able to significantly modify their growth pattern to give priority to the activation of defense responses. The redistribution of cell cycle activity and the control of cell expansion are key events in the acclimation of plants to stress. In this work we show that exposure of tobacco BY-2 cells to heat stress caused a significant reduction in growth: firstly a decrease in cell division and successively an inhibition of cell expansion occurred. The inhibition of mitosis seems to be due to the arrest of cell cycle progression at the

G2-M checkpoint. Indeed, a decrease in cyclin A, cyclin B and CDKB expression and an increase in Cyclin D3 expression were observed. At the same time the inhibition of cell expansion was accompanied by an under-expression of expansin-8, a protein involved in the regulation of cell wall flexibility. Interestingly, changes observed in the methylative state of the promoters of Cyclin D3 and expansin-8 were well correlated with the alteration in their expression, suggesting that an epigenetic control of these genes could be involved in the inhibition of growth observed under heat stress.

O19.3
Plant response mechanisms to drought and insect pest attack: signals from the roots to the leaves.

S. Pasqualini[1], G. Salerno[2], F. Frati[2], G. Marino[3], M. Centritto[4], F. Loreto[3], C. Brunetti[3], L. Ederli[1]
[1]Dept Chemistry, Biology & Biotechnology Univ. Perugia, Italy, [2] Dept Agricultural, Food & Environmental Sciences, Univ. Perugia, Perugia, Italy, [3] Inst Plant Protection - CNR Sesto Fiorentino, Florence, Italy, [4] INVALSA - CNR- Sesto Fiorentino, Florence, Italy

Many environmental stresses suffered by roots can influence the production of the aerial parts of plants, and thus reduce biomass and productivity. In addition, root stress can alter plant defenses against biotic stress, with consequences on plant fitness and ability to communicate with other organisms. Among root stresses, water stress is important at the global level. Limited availability of water is a problem throughout Europe, which is likely to worsen due to climate change. Often, plants have to balance their interactions between abiotic and biotic stress. This study investigates whether water stress in Vicia faba affects feeding of the herbivore Nezara viridula and of its egg parasitoid, Trissolcus basalis. We present here data on the signaling molecules ABA, SA and H_2O_2, and measures of captured BVOCs in terms of attractiveness to the insect parasitoid. Understanding of these aspects will help to define the mechanisms of control against these attacks by herbivores through the egg parasitoids, also considering sustainable pest management., This study is supported by grant 2013-0049-021 from the Fondazione Cassa di Risparmio di Perugia, and from MIUR (PRIN 2010-2011 "PRO-ROOT").

O19.4
Addressing molecular and cellular response mechanisms induced by salt stress in two contrasting salt sensitivity varieties of Italian rice (*Oryza sativa* ssp. Japonica)

E. Formentin, C. Sudiro, E. Barizza, M. Zottini, F. Lo Schiavo
Dept Biology, Padua Univ., Padova, Italy

Soil salinity affects crop production on over a quarter of all agricultural land on Earth, in particular in coastal areas. In the frame of a research project of national interest (RISINNOVA), salinity response was analysed in two Italian rice varieties (Baldo and Vialone Nano) selected for contrasting salt sensitivity. Among components involved in the signaling pathway induced by salt, the role of nitric oxide was investigated both in cultured cells and in plant roots. At molecular level, an RNA sequencing analysis, aimed to compare the transcriptional profile of the two cultivars, was performed. In the more sensitive cultivar Vialone Nano, a clear down-regulation of genes involved in chlorophyll biosynthesis along with leaf chlorosis is observed, indicating an early induction of senescence events. Moreover, the up-regulation of heat-shock protein coding genes indicates that Na+ reached toxic concentrations in V. Nano leaves leading to a massive protein misfolding. Baldo leaves, instead, respond to salt stress putting in place the activation of specific responses to water deficit in coordination with ROS detoxification pathways, so resulting in being more successfully., Supported by Progetto AGER, grant n° 2010-2369

O19.5
Unraveling the redox regulation and structure of Calvin-Benson cycle enzymes from *Chlamydomonas reinhardtii*: from proteomic data to *in vitro* studies

M. Zaffagnini[1], S. Morisse[2], M. Pasquini[1], L. Michelet[2], C. H. Marchand[2], F. Sparla[1], F. Francia[1], S. Fermani[3], S. D. Lemaire[2], P. Trost[1]
[1]Department of Pharmacy and Biotechnology (FABIT), University of Bologna, Bologna, Italy, [2]Institut de Biologie Physico-Chimique, CNRS, Université Pierre et Marie Curie, Paris, France, [3]Department of Chemistry "G. Ciamician", University of Bologna, Bologna, Italy

In oxygenic photosynthetic eukaryotes, the Calvin-Benson cycle (CBc) was previously indicated as a redox regulated process (1). Notably, 4 out 11 enzymes are well-established thioredoxin target regulated through dithiol/disulfide exchange reactions. Recently, proteomic studies suggest that all CBc enzymes may withstand redox regulation *via* disulfide bond formation (S-S) and other thiol-based modifications named glutathionylation (S-SG) and nitrosylation (S-NO) (1). The aim of this study was to analyze the molecular mechanisms underlying the redox regulation of CBc enzymes from *C. reinhardtii*. The redox analysis was carried out on recombinant proteins (WT and cysteine variants) by means of biochemical, biophysical and structural approaches (activity assays, redox and thiol titrations, mass spectrometry, CD, DLS, and crystallography). Taken together, our results confirmed that most of the CBc enzymes are subjected to multiple redox control and this regulation is dependent on both light and stress conditions (*i.e.* TRX redox state and ROS/RNS, respectively), enlightening the intricate network of redox regulation mechanisms controlling the CBc. *(1) Michelet et al, 2013, FIPS 4: 1-21*

20 - Plant Nutrition

P20.1
Nitrate control of nodule formation in Lotus japonicus: functional characterization of a low affinity nitrate transporter

L. M. Alves[1], S. Leran[2], V. T. Valkov[1], A. Rogato[1], B. Lacombe[2], M. Chiurazzi[1]

[1]Institute of Biosciences and Bioresources, CNR, Via P. Castellino 111, 80131, Napoli, Italy, [2]Institute de Biologie Intègrative des Plantes, C. Grignon, CNRS/INRA/UM2/SupAgro, Place Viala, 34060, Montpellier, FR

Plant adaptations include the capacity to respond to changes of the nutrient availability in the soil by modulating their root system developmental plan. NO_3^- is able to trigger signaling pathways modulating systemically, or locally, lateral root development and in legumes nitrate availability strongly affects nodule initiation. We are investigating the role of *LjNPF4.1*, a nitrate-inducible low affinity transporter of the *L. japonicus* NPF family in the control of the nodule formation pathway. The profile of expression in roots suggested a role on root uptake of external nitrate. The nitrate transport activity was confirmed in Xenopus oocytes injected with the LjNPF4.1 cRNA. Furthermore, a tagged knock out *Ljnpf4.1* mutant was isolated showing a 70% reduction of nitrate uptake in planta in a high concentration range. This phenotype is associated to significant reduction of the shoot growth parameters when compared to wild type plants. Interestingly, we also observed a root developmental phenotype with a specific deficiency on the root elongation pathway. We'll present data on symbiosis-related phenotype of the knock out mutant.

P20.2
Potassium deficiency and drought stress in grapevine cultivars

E. Oddo[1], L. Abbate[2], S. Inzerillo[1], M. Calabrese[1], F. Carimi[2], A. Motisi[2], M. Sajeva[1], A. Nardini[3]

[1]Dept STEBICEF,University of Palermo,via Archirafi 20,Palermo,Italy, [2]Institute of Bioscences and BioResources-CNR,Corso Calatafimi 414,Palermo,Italy, [3] Dept Scienze della vita,University of Trieste,via Giorgieri 10,Trieste,Italy

Potassium availability affects drought response in plants trhough severalmetabolic roles,among which stomatal regulation,cell growth and xylem hydraulics.*Vitis Vinifera*L. is a highly valuable crop and several genotypes have been selected during its millennial cultivation. Varieties show differences in their adaptability to stress conditions,making them more or less suitable to certain climatic and edaphic conditions. the varieties cultivated in Sicily are characterized by high variability. We investigated the response of two Sicilian cultivars (Nero d'avola and Catarratto) to otassium deficiency and drought stress. Two year old grafted plants were grown in agriperlite,with ore without potassium in the fertigation solution,and subjected to moderate drought stress by suspending irigation for 6-8 days. Potassium content of xylem sap,leaf and root tissues ere measured with an ion selective electrode. Changes in stomatal conductance,plant traspirantion and hydraulic conductance were compared between genotypes and treatments,in order to gain information for the development of optimal fertigation practices and selection of the most drought tolerant varieties

P20.3
The interplay between photorespiration and iron deficiency: a preliminary investigation.

F. M. Casiraghi, G. Zocch

Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Milano

Iron (Fe) is an essential element for living organisms being a cofactor of many metabolic processes. The most noticeable effect of Fe-deficiency in leaves is a marked chlorosis caused by a lack in chlorophyll biosynthesis which results in reduction of photosynthesis and induction of oxidative stress. Photorespiration can be considered as a cycle which helps to defend plants from those impairments., Metabolic characterization of *Cucumis sativus* L. plants grown at different Fe concentration was performed to investigate the interplay among Fe deficiency and photorespiration. *In vivo* analysis of photosynthetic and chlorophyll fluorescence parameters suggest limitations in photosynthesis and induction of other energy dissipation processes. The activity of Fe-dependent enzymes involved in photorespiration was lower in Fe-deprived plants., Purification of the peroxisomal fraction to carry out Western Blot analysis of enzymes belonging to photorespiration was undertaken. Our data suggest that the imbalance induced by Fe deficiency may be balanced by increased rate of photorespiration. Future prospect will be the purification and characterization of Fe-deficient chloroplasts and mitochondria.

P20.4
Molecular characterization of two cation transporters of *Arabidopsis thaliana*

S. Piasentin, G. DalCorso, A. Furini

Dipartimento di Biotecnologie, Università degli studi di Verona, strada le Grazie 15, 37134 Verona

CTP (**C**ation **T**ransporter **P**rotein) genes code for a widely distributed class of proteins involved in microelement absorption and distribution in plant tissues. Two still partially characterized proteins, CTP1 and CTP2, are putatively involved in the transport of divalent cationsand are able to transport Mn and Zn in yeast. Phenotypical analyses on single and double mutants (dm) for CTP1 and CTP2 of *Arabidopsis thaliana,*show a mild reduction in root length in the dm grown in deficiency or excess of Mn and Zn. In standard condition the dm displays no obvious phenotypes except for imbalances in metal content. *AtCTP1*promoter drives GUS expression in the vascular tissue, while *AtCTP2*promoter in the entire root tissue; in flowers and siliques the transcription of both genes is localized in the style region. Moreover, these results correlate with those obtained in the Real-time based analyses that showed for both genes the highest trascription in flowers and siliques and the lowest in shoots. To further characterize both genes, the analysis of over-expressing lines and the sub-cellular localization of the two transporters will be performed.

P20.5
Comparative study on physiological and transcriptional responses to iron and phosphorus deficiency in roots of *Lupinus albus* L.

S. Venuti[1], L. Zanin[1], L. Zanon[1], N. Tomasi[1], R. Pinton[1]

Dept Agricultural and Environmental Sciences, University of Udine, Udine, Italy

White lupin (*Lupinus albus* L.) has developed a highly efficient strategy to mobilize nutrients, in particular P and Fe, from soils. This strategy is based on the modification of the root architecture with the formation of the so-called "cluster roots" that are able to release large amounts of exudates. While the P deficiency response is well characterized, the mechanisms involved in the release of root exudates in Fe deficiency conditions are still barely known., White lupin plants were grown under P or Fe deficiency for 5 weeks and then the physiological adaptations were evaluated providing soluble or poorly soluble radio-labeled sources of Fe or P for up to 24 hours (Fe-EDTA or Ferrihydrite and KH_2PO_4 or Vivianite, respectively). The data showed that Fe-deficient plants accumulated quickly Fe from Fe-EDTA in roots while Fe acquisition from Ferrihydrite was slower. Root apices of Fe-deficient plants were the most efficient tissues in acquiring Fe from Ferrihydrite, while the highest capacity to acquire P occurred in cluster roots of P-deficient plants. The transcriptomic data are being analyzed via RNAseq., *Work supported by a grant from Italian F.I.R.B.*

O20.1
Sentinel plants to improve sulfur use efficiency: living instruments for nondestructive analysis

M. Maghrebi[1], C. Lancilli[1,2], A. Ferri[1], F. F. Nocito[1], G. A. Sacchi[1]
[1]Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano, [2]Istituto di Biologia e Biotecnologia Agraria (IBBA), Consiglio Nazionale delle Ricerche

Developing bioassays based on the use of sentinel plants to quickly determine nutrient bioavailability and/or crop nutritional status may represent a reliable and efficient strategy to obtain valuable, timely and low-cost information about changes in nutrient availabilities and nutritional requirements in a crop system., Two Arabidopsis bioindicators - carrying the GUS reporter gene under the control of two portions of the intergenic region between *At1g12030* and *At1g12040* - have been characterized for their potential ability to provide information about the sulfur nutritional status of the plant and/or the sulfate concentration in the growing medium. For this purpose, the two lines were grown in agar plates under a continuous sulfate gradient ranging from 0 to 150 µM in order to describe the growth of both roots and shoots as a function of sulfate external concentration and to determine the critical concentration of sulfate (i.e. the minimum concentration of sulfate necessary to achieve maximum biomass) in the growing medium., The main results indicate that both the lines are able to correctly indicate the critical concentration of sulfate in the external medium also in the presence of interfering metal ions (Cd^{2+}) able to increase the plant metabolic demand for sulfur. Moreover, the two intergenic regions are suggested as bi-directional promoters able to control the expression of two flanking genes under sulfur limitation.,

O20.2
Modulation of root glutamine synthetase isoforms and plant amino acid balance in response to nitrogen nutrition in maize

B. Prinsi, L. Espen
Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Italy

Glutamine synthetase (EC:6.3.1.2) catalyzes the first step in mineral nitrogen (N) assimilation. In maize (*Zea mays* L.) five cytosolic (GS1) isoforms and one plastidic (GS2) enzymes are known. This study was focused on plants exposed by hydroponic systems to different inorganic N source: NO_3^-, NH_4^+ or both. The proteomic characterization of root profiles put in evidence that, while the GS2 was exclusively involved in NO_3^- assimilation, the GS1 isoforms were differently and specifically regulated in response to both nutrients. At the same time, the LC-ESI-MS analysis of amino acid speciation in root, xylem and leaves highlighted as the assimilatory pathways were deeply influenced by N sources. In particular, while NO_3^- assimilation was almost equally distributed in the two apparatus, the NH_4^+ organication was predominantly located in the roots. This compartmentalization seemed to be allowed by a modulation of whole plant metabolism. In fact, the increased accumulation of asparagine in root was related to an increase in xylem translocation of glutamine, which in leaves appeared recycled in alanine and glutamate to cope with the higher request of carbon skeletons.

O20.3
Systematic characterization of members of the *L. japonicus* NPF family up-regulated in symbiotic nitrogen fixation nodules. What's their role on nodule functioning?

V. T. Valkov[1], A. Rogato[1], L.M. Alves[1], S. Leran[2], D.A. Carbone[1], L. Duplice[1], B. Lacombe[2], M. Chiurazzi[1]
[1]Institute of Biosciences and Bioresources, CNR, Via P. Castellino 111, 80131, Napoli, Italy, [2]Institute de Biologie Intègrative des Plantes, C. Grignon, CNRS/INRA/UM2/SupAgro, Place Viala, 34060, Montpellier, Fance

Nitrate plays an important role as regulator of legume nodules activity but the mechanism through which this action takes place is still controversial. Apparently nitrate targets the formation of the nitrogenase complex and ATP generation. Nitrate transporters may be involved in this network supporting the nodule functioning. Besides, the versatility of these transporters for different substrates may also suggest a direct role in the transport of carbon sources essential for rhizobia. In silico search identified 70 members of the *L. japonicus* NPF family. Experiments conducted by our group as well as a screening of *L. japonicus* microarrays and Expression Atlas data allowed to identy a sub-group of eight Lotus NPF members specifically induced in nodular tissue. Part of the characterization of these up-regulated members is performed in heterologous systems: i) complementation analysis in known *E. coli* and yeast transport mutants; ii) biochemical characterization in injected Xenopus oocytes. The *in planta* analysis is based on the spatial localization of the promoter activities and a reverse genetic approach with Lotus LORE1 lines tagged in most of these transporter coding genes.

O20.4
Transcriptional and physiological aspects of Fe deficiency response in roots of *Zea mays*

L. Zanin[1], S. Venuti[1], N. Tomasi[1], A. Zamboni[2], S. Cesco[3], Z. Varanini[2], R. Pinton[1]
[1]Dept Agricultural and Environmental Sciences, University of Udine, Udine, Italy, [2]Dept Biotechnology, University of Verona, Verona, Italy, [3]Faculty of Science and Technology, Free University of Bolzano, Bolzano, Italy

Plants react to iron (Fe) deficiency using different adaptive strategies. Under limited Fe availability maize, an economically important crop and a model species for *Strategy II* plants, improves Fe acquisition via the release of phytosiderophores (PS) into the rhizosphere and the subsequent uptake of the Fe-PS complex into root cells. In this work, microarray analysis identified 376 genes differentially modulated by Fe-deficiency in roots (289 up- and 87 down-regulated). Of particular interest, genes coding for many transcription factors and for the synthesis and release of PS were found induced by the nutritional stress. The capacity of maize plants to respond to the Fe-deficiency was further evaluated exposing roots to soluble or poorly soluble Fe-sources for up to 24 hours. Beside real time RT-PCR analyses, ^{59}Fe uptake experiments showed that the mechanisms involved in Fe acquisition were induced by the nutritional stress; however the downstream pathway involved in the translocation and distribution of the micronutrient within the plant were not yet activated in Fe-deficient plants. *Work supported by a grant Italian F.I.R.B.*

O20.5
Plant growth promotion effects of endophyte community associated to *Vitis vinifera* cv. Glera

E. Baldan[1], E. Barizza[1], S. Nigris[1], S. D'Alessandro[1], E. F. Figueredo[1-3], A. Squartini[2], B. Baldan[1], M. Zottini[1]
[1]Dipartimento di Biologia, via U Bassi 58/B, 35131 Padova, [2]Dipartimento DAFNAE - Department of Agronomy Food Natural Resources Animals and Environment, Viale dell'Università 16, 35020 Legnaro (PD), [3]CAPES Universidade Federal Rural de Pernambuco - UFRPE/UAG - Pernambuco, Brasil

Endophytic bacteria may stimulate host plant growth through any of several possible mechanisms including biological control, induced systemic resistance to plant pathogens, phytohormone production and improvement of nutrient and water uptake. Some of these organisms can be utilized in agricultural and horticultural practices for the purpose of transplant protection against diseases, improvement of establishment and overall performance. It may also improve plant performance in stress environments and consequently enhance yields. In this work we report on the isolation and characterization of culturable bacterial endophytes colonizing Glera grapevine cv. growing in North of Italy (Veneto, Italy). In six different vineyards within the area, 381 culturable strains were isolated and molecularly characterized by Amplified Ribosomal DNA Restriction Analysis (ARDRA). Different biological activity of these endophytes have been evaluated, such as auxin production, phosphate solubilization activity and antifungal properties. Plant growth promoting activity has been tested in *in vitro* grown grapevine plants and in Arabidopsis plants harboring specific reporter-genes.

21 - Protein Synthesis, Degradation and Homeostasis

P21.1
The role of Drosophila pseudouridine synthase component of H/ACA snoRNPs in compensatory proliferation and tissue remodeling

A. Di Giovanni, L. Cassese, L. F. Iannucci, A. Petrizzo, R. Vicidomini, M. Furia
Department of Biology, University of Naples "federico II", Complesso Univeristario di monte Sant'Angelo, via Cinthia I-80126

Eukaryotic pseudouridine synthases belong to a protein family highly conserved from Archaea to man, and are essential components of the H/ACA snoRNP complexes involved in ribosome biogenesis and pseudouridylation of target RNAs. The striking evolutive conservation of these proteins makes *Drosophila melanogaster* an useful model system to investigate the variety of functions currently attributed to H/ACA snoRNPs. We thus investigated the role of the *Nop60b/minifly* (*mfl*) gene -that encodes the fly pseudouridine synthase- in tissue homeostasis, by regionally manipulating its activity by RNAi*in vivo*. We found that *mfl* silencing triggers both apoptosis and compensative proliferation, as occurring in typical regenerative phenomena. Moreover, disruption of the apical/basal polarity of β-catenin and de-polymerization of F-actin filaments was observed. These data indicate that *mfl* silencing induces concomitant cell death and proliferation accompanied by a dramatic cytoskeletal remodelling, pointing toward a central role of eukaryotic pseudouridine synthases in tissue homeostasis and regenerative processes.

P21.2
Structural and functional characterization of crested porcupine (*Hystrix cristata* L.) and reindeer (*Rangifer tarandus* L.) myoglobins

A. M. A. Di Giuseppe[1], S. Ragucci[1], V. Severino[1], A. Chambery[1], A. Di Maro[1]
[1]Dept of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Caserta, Italy

Myoglobin (Mb) is a hemoprotein, expressed in cardiac myocytes and oxidative skeletal muscle fibers, that reversibly binds O_2 by its heme b group. Mb is an excellent model protein for its role in several human diseases, for understanding the structure/function relationships in proteins and also for its importance in the food industry since it determines the meat color., Our research group is studying since long time Mbs isolated from several sources for their potential use as molecular markers for the detection of undeclared species in raw meat., In this work, the primary structure of two novel myoglobins isolated from crested porcupine (Hystrix cristata L.) and reindeer (Rangifer tarandus L.) meats was determined by using a combined approach based on MALDI-TOF MS and LC-ESI MS/MS. The strategy allowed the rapid elucidation of the primary structure of both Mbs. The experimental molecular masses determined by ESI Q-TOF on the native crested porcupine and reindeer Mbs of 16867.25 Da and 16924.06 Da were found to be in good agreement with the theoretical molecular masses deduced from the amino acid sequence (16867.38 Da and 16923.36 Da, respectively). Preliminary studies were also performed for evaluating Mbs autoxidation rates.,

P21.3
Molecular mechanisms of tonoplast protein degradation

C. Lancilli, C. Perico, M. Maîtrejean, D. Mainieri, E. Pedrazzini, A. Vitale
Istituto di Biologia e Biotecnologia Agraria, CNR, Milano, Italy

Protein turnover is fundamental both for development and cellular homeostasis. We are studying the possible mechanisms involved in the degradation of a tonoplast protein, the Arabidopsis potassium channel TPK1, previously shown as degraded upon internalization into the vacuole. In order to investigate if TPK1 degradation occurs via a multivesicular bodies mediated pathway, or via a direct pathway, where the internalization event would occur directly at the vacuolar membrane, we are using two different approaches, based on site-directed mutagenesis of TPK1 lysine codons or on specific traffic inhibitors. First results of pulse-chase experiments on tobacco protoplasts expressing wild-type or three lysine deprived TPK1-GFP constructs showed no differences in the TPK1 turnover, suggesting that the ubiquitination of TPK1 does not occur, or at least does not involve the lysine residues we supposed. Site-directed mutagenesis of other TPK1 lysine codons is in progress, as well as in vivo treatments with tyrphostin A23 and wortmannin of Arabidopsis plants overexpressing TPK1-GFP., Supported by Italian Ministry of Education, Universities and Research (PRIN2010CSJX4F).

P21.4
The dual role of the p300 acetyl transferase protein on ΔNp63α stability

A.V. Tramontini, M. Restelli, E. Molinari, L. Guerrini
Dept of Biosciences, Università degli Studi di Milano, 20133 Milano Italy

ΔNp63α is a member of the p53 transcription factor family and plays a crucial role in epithelial development. Natural dominant ΔNp63α mutations causes improper development of skin, teeth, mammary glands and limbs. The p53 protein is acetylated and positively regulated by the histone acetyl transferase protein p300. The p300 N terminal domain, p300_1-595, has been demonstrated to have an E3 ubiquitin ligase activity towards p53. We have demonstrated that transcriptional activation of ΔNp63α is induced by p300 acetylation upon FGF8 treatment both *in vitro*and in *ex vivo*limb bud cultures. Here we report the effect of overexpression of the p300 N-terminal fragment (p300_1-595) on ΔNp63α. The p300_1-595 fragment induced degradation of the ΔNp63α protein, but the double ΔNp63α_K494R-K505R mutant was resistant to this degradation, suggesting that these lysines could be targets of p300_1-595 ubiquitination. Preliminary data showed that the p53 protein is implicated in this degradation event, because the degradation of ΔNp63α by p300_1-595 was abolished in a cell line not expressing p53. Elucidation of this pathway may help to identify the molecular mechanisms regulating ΔNp63α stability.

O21.1
Structural perturbations in hereditary amyloidosis: the case of two variants of apoA-I

F. Itri, R. Del Giudice, A. Arciello, R. Piccoli, D. M. Monti
Dept. of Chemical Sciences, Federico II Univ., Naples, Italy

Several naturally occurring mutations in the ApoA-I gene induce in vivo fibril formation causing systemic amyloidosis. A physicochemical characterization of recombinant ApoA-I amyloidogenic variant Leu75 with Pro (L75P) and Leu174 with Ser (L174S) was performed. At physiologic-like conditions (pH 7.4) both variants undergo a conformational transition to a β-sheet rich structure, confirmed by increase in thioflavin T fluorescence over-time. By fluorescence analyses we demonstrated that both variants have an increased hydrophobic surface. In particular, L174S variant was found to have a compact structure similar to that of wt-ApoA-I, whereas L75P variant was found to have a more relaxed tertiary structure. Interestingly, both variants show a decreased chemical and thermal stability, with L174S the most susceptible to perturbations. We also found that both variants are less protected to limited proteolysis with respect to wt-ApoA-I. Our results clearly indicate that amyloidogenic ApoA-I variants are more susceptible to environmental changes and are more prone to aggregate when destabilizing conditions occur.

O21.2
A single-chain variable fragment antibody against Z alpha1-antitrypsin prevents intracellular polymerisation

A. Ordóñez[1], J. Pérez[2], L.V. Tan[1], J.A. Dickens[1], N. Motamedi-Shad[3], J.A. Irving[3], I. Haq[3], U. Ekeowa[1], S.J. Marciniak[1], E. Miranda[4], D.A. Lomas DA[3]
[1]Dept. of Medicine, University of Cambridge, Cambridge Institute for Medical Research, UK, [2] Dept. of Cell Biology, Genetics and Physiology, University of Malaga, Spain, [3] Wolfson Institute for Biomedical Research, University College London, London, UK, [4] Dept. of Biology and Biotechnologies 'Charles Darwin' and Pasteur Institute - Cenci Bolognetti Foundation, Sapienza University of Rome, Italy

Polymerisation of mutant Z alpha1 antitrypsin (AAT) underlies AAT deficiency, in which mutant protein accumulates within the endoplasmic reticulum (ER) of hepatocytes predisposing to liver disease, while the lack of active AAT leads to lung emphysema. Here we explored the therapeutic potential of antibody-based strategies in the field of AAT deficiency., Using standard monoclonal antibody (mAb) procedures, we produced mAbs against monomeric Z AAT and through a novel screening assay we identified mAb 4B12 that robustly blocked polymerisation of Z AAT in vitro. MAb 4B12, as full-length or Fab region, blocked polymer formation at a 1:1 molar ratio, forming a complex that could be identified in non-denaturing PAGE, and which retained 36% of the inhibitory activity of Z AAT. We then generated an ER-targeted intrabody molecule based on the single-chain variable fragment (scFv) of mAb 4B12, which efficiently prevented polymerisation when co-expressed with Z AAT in COS-7 cells., In conclusion, mAb 4B12 blocks the polymerisation of Z AAT and can be used to study this process in vitro, while its intrabody version reduces polymerisation in cells, opening up new therapeutic interventions.

O21.3
Proteostasis of the A.thaliana TPK/KCO channels

M. Bertolini[1], D. Mainieri[1], C. Lancilli[1], C. Perico[1], L. Carraretto[2], A. Vitale[1], I. Szabò[2], E. Pedrazzini[1]
[1]Istituto di Biologia e Biotecnologia Agraria, CNR, Milano, Italy, [2]Dipartimento di Biologia, Univ. Padova, Italy

Plant channels control the vectorial transport of fluid, solutes and electrolytes. To perform these functions, channels must be targeted, sorted and retained at the appropriate membrane and be subjected to regulated turnover. We are studying the proteostasis of the five tandem-pore (TPK1-5) and the single one-pore (KCO3) K+ channels of A. thaliana. Transient or transgenic expression of GFP fusions, or localization of endogenous proteins showed localizations at the tonoplast, plasma membrane, thylakoid membranes, as well as unidentified intracellular structures, depending on the channel. With the aim of clarifying the situation, we have first compared in silico the N-termini of TPK/KCO. We found that AtTPK3, 5 and 2 contain N-terminal regions with similar features and putative chloroplast/mitochondrial presequences. To verify in vivo our in silico prediction, TPK/KCO-N-termini::GFP fusions have been expressed. We also identified a putative PDZ-binding motif of class 1 at the C-terminus of KCO3. The role of this motif in KCO3 turnover has been analysed. Supported by Italian Ministry of Education, Universities and Research (PRIN2010CSJX4F)

O21.4
HGA-induced aggregation and fibrillogenesis of amyloidogenic proteins: implications in alkaptonuria

D. Braconi, L. Millucci, G. Bernardini, S. Gambassi, M. Laschi, M. Geminiani, L. Ghezzi, A. Bernini, O. Spiga, N. Niccolai, A. Santucci
Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Siena (Italy)

Alkaptonuria (AKU) is a rare disease with no therapy due to homogentisic acid (HGA) accumulation and production of an ochronotic pigment responsible for organ damage. We previously found that AKU is a secondary AA amyloidosis, and that AKU cells show aberrant expression of proteins involved in amyloidogenesis, folding and proteases suggesting increased protein oxidation/aggregation. Here we found that HGA can significantly accelerate the aggregation and fibrillogenesis of Aβ(1-42), transthyretin, α-synuclein and SAA, as observed by spectrophotometric methods, leading to the production of Congo Red positive aggregates up to formation of oligomers, protofibrils and fibrils, indicating a role for HGA in SAA-amyloid production in AKU. Mass spectrometry and in silico studies on HGA-SAA interaction confirmed experimental data suggesting the involvement of SAA amino acid residues critical for heparan sulfate binding and aggregation. Helping the clarification of AKU pathogenesis and related amyloidogenesis, our results could lay the basis for appropriate pharmacological interventions for AKU and other types of amyloidoses., Acknowledgements: Financial support from TLSF-Orphan0108 and Telethon GGP10058.

O21.5
An evolutionary model for protein body formation in the endoplasmic reticulum of cereal endosperm cells

D. Mainieri, F. Morandini, M. Maîtrejean, A. Saccani, E. Pedrazzini, A. Vitale
Istituto di Biologia e Biotecnologia Agraria, CNR, Milan, Italy, EU

The seed storage proteins present in all plants accumulate in storage vacuoles. Prolamins, which are the major seed storage proteins in cereals and are present only in these plants, instead accumulate within the endoplasmic reticulum (ER) lumen as very large insoluble polymers held by disulfide bonds, termed protein bodies. The model prolamin 27 kD γ-zein of maize contains seven cysteine residues involved in interchain bonds. We show that progressive substitution of these amino acids with serine residues leads to similarly progressive increase in solubility and availability to traffic from the ER along the secretory pathway. Total substitution results in very efficient secretion, whereas the presence of a single cysteine is sufficient to promote partial sorting to the vacuole via a pathway that is sensitive to brefeldin A and wortmannin, similarly to the normal traffic pathway of vacuolar storage proteins. We propose that the mechanism leading to accumulation of prolamins in the ER is a further evolutionary step of the one responsible for accumulation in storage vacuoles. Supported by the FILAGRO Project of CNR-Regione Lombardia.

22 - Environmental and Molecular Mutagenesis

P22.1
Titanium dioxide nanoparticles. A potential use in polluted environment remediation

M. Bernardeschi, P. Guidi, V. Scarcelli, P. Lucchesi, C. Ghezzani, M. Nigro, G. Frenzilli
Department of Clinical and Experimental Medicine, University of Pisa, Via Volta 4, 56126 Pisa, Italy

The employment of nanoparticles (NPs), particularly metallic ones, such as titanium dioxide, has been recently suggested as a good alternative in polluted environment remediation. The efficiency and safety of NPs are obviously needed, since the powders should be inert with respect to the environment but able to remove heavy metals and organic pollutants, if present. In the present work, the potential genotoxicity exerted by TiO_2 nanoparticles has been *in vitro*and *in vivo*tested on the sentinel species, the marine mussel (*Mytilus galloprovincialis*)and the fish sea bass (*Dicentrarchus labrax*).Comet assay and Diffusion assay, two fast and sensitive genotoxicity tests able to assess DNA damage, were applied. Preliminary *in vitro*results after *M. galloprovincialis*gills biopsie exposure to mesoporous titanium dioxide NPs (25nm) showed a lack of genotoxic effects. Consequently, a co-exposure with crude oil was *in vivo*performed using *D. labrax*.The co-treatment with meso-$TiO_2$and crude oil highlighted a decrease in genotoxic effect exerted by the pollutant alone. More testes are needed to confirm these results, which suggest a good nano-TiO_2safety and remediation capacity.

P22.2
Effects of copper and nickel on protamine-like and on DNA oxidative damage in *Mytilus galloprovincialis*

F. Caccavale, F. Pollice, M. Restaino, G. Speranza, S. Avagnano, D. Drongitis, G. Guerriero, L. Fucci, J. Ausió[2], M. Piscopo[1]
[1]Dept of Biology, University of Naples Federico II, Italy, [2]Dept of Biochemistry & Microbiology, University of Victoria, British Columbia, Canada

In sperm cells, DNA is complexed with sperm nuclear basic proteins (SNBPs) to form chromatin. Although SNBPs protect DNA from a variety of potentially dangerous reactive species, such as hydroxyl radicals (•OH), chromatin packaging doesn't protect DNA from metal ion-dependent damage in the presence of hydroxyl radicals. We report the effects induced by Cu^{2+} and Ni^{2+} ions on DNA and *Mytilus galloprovincialis'* protamine-like II and III. Protamine-like III acquires proteinase k digestion resistance in the presence of copper and not in the presence of nickel; PLII, in contrast with PLIII, is sensitive to proteinase k digestion in presence of both metals. Further, copper promotes hydrogen peroxide damage of DNA also in the presence of PL-types while nickel promoting PL-types aggregation enhances DNA compactness and protection. Nickel in fact, in contrast to copper, increases PLII and PLIII DNA binding affinity. All these effects can lead an anomalous chromatin packaging and, therefore, a reduction of *Mytilus galloprovincialis'*reproductive fitness and of other organisms exposed to the same toxicants.

P22.3
Influence of titanium dioxide nanoparticles on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and cadmium genotoxicity in the marine fish European sea bass (*Dicentrachus labrax*). A Trojan horse effect?

P. Guidi[1], M. Bernardeschi[1], V. Scarcelli[1], C. Ghezzani[1], L. Rocco[2], I. Corsi[3], G. Frenzilli[1], M. Nigro[1]
[1]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [2]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), Seconda Università di Napoli, Caserta, Italy, [3]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy

Due to the large production and growing application of titanium dioxide nanoparticles (n-TiO_2) their occurrence in the marine environment is expected to represent an actual risk for biota. Based on their physical-chemical properties, a potential interaction with classical pollutants might take place affecting their uptake and toxicity in marine organisms. We investigated the influence of n-TiO_2 on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and on Cd genotoxicity for the marine fish European sea bass *Dicentrarchus labrax* during 7 days *in vivo* exposure. Comet assay, Micronucleus (MN) test and Random Amplified Polymorphisms DNA (RAPD)-PCR were used to study genotoxicity. Titanium dioxide nanoparticles were found to reduced DNA damage caused by 2,3,7,8-TCDD and Cd in peripheral nucleated erythrocytes. n-TiO_2either alone and in combination with 2,3,7,8-TCDD caused a significant increase in chromosomal damage shown by altered MN frequency. Results show a genotoxic potential of n-TiO_2 in fish cells but do not seem to indicate a synergistic effect of n-TiO_2 with the selected classical pollutants of marine environments.

P22.4
Cadmium effects on protamine-like and on DNA oxidative damage in *Mytilus galloprovincialis*

F. Pollice, F. Caccavale, G. Speranza, S. Avagnano, D. Drongitis, G. Guerriero, L. Fucci, J. Ausió[2], M. Piscopo[1]
[1]Dept of Biology, University of Naples Federico II, Italy, [2]Dept of Biochemistry & Microbiology, University of Victoria, British Columbia, Canada

Heavy metal pollution has become one of the most serious environmental problems. They can make DNA damage, due to their ability to form reactive oxygen species, causing mutations in human cells which may cause tumors. In order to monitor sea levels of heavy metals are used specific bioindicators like *Mytilus galloprovincialis*whose sperm chromatin is organized by three protamine-like (PLII, PLIII and PLIV), which belong to sperm nuclear basic proteins. We report the effects of Cd^{2+}on DNA, PLII and PLIII. Both PLII and PLIII acquired a slight proteinase K digestion resistance in the presence of metal. We also analyzed the self-association ability of PLII and PLIII Cd^{2+}mediated. Turbidity assay showed that cadmium caused PLII but not PLIII aggregation, while glutaraldehyde produced a reduction of self-association ability for both proteins. Further, Cd^{2+} induced a decreasing of PLII DNA binding affinity but an increasing for PLIII and promoted H_2O_2 DNA damage even in the presence of Protamine-like. Those effects may result in an incorrect chromatin packaging that could decrease *Mytilus galloprovincialis'* fitness and that of other marine species exposed to this polluting agent.

P22.5
Effect of crystal habit on silica particle genotoxicity in human and murine cell lines

V. Scarcelli, P. Guidi, M. Bernardeschi, P. Lucchesi, C. Ghezzani, M. Nigro, G. Frenzilli
Department of Clinical and Experimental Medicine, Pisa Univ., Pisa, Italy

Crystalline silica inhaled from occupational sources has been classified by IARC as carcinogenic to humans; amorphous silica could not be classified for carcinogenicity as evidences from epidemiological and experimental studies are still insufficient. However, the genotoxic potential of crystalline silica is still debated because of the "variability of silica hazard", often related with the features of particle surface. This study is aimed to assess if crystal habit is responsible for genotoxicity of silica powders. Pure quartz (crystalline) and vitreous silica (amorphous), sharing the same surface features, were used for an *in vitro* approach. Human pulmonary epithelial (A549) and murine macrophages (RAW264.7) cell lines, were selected for investigation. Genotoxicity was evaluated by Comet assay and Micronucleus test, cytotoxicity was tested by the Trypan blue method. Cells were treated with 0, 5, 10, 20, 40, 80

μg/cm^2 of flours for 4 and 24 h. VS showed no cyto- and genotoxicity effects in the two cell lines used; quartz induced increased cell death and DNA damage in RAW264.7 but not in A549 cells. Results show crystal habit as crucial for biological hazard of silica particles.

P22.6
Characterisation of RNA-editing deficient DNA-editing proficient mutants of the RNA editing enzyme APOBEC1

F. Severi, S. Sarti, S. Conticello
Core Research Laboratory, Istituto Toscano Tumori - AOU Careggi, Firenze, Italy

The AID/APOBECs are cytosine deaminases acting in the context of nucleic acids. APOBEC1 is the catalytic subunit of a complex that edits the C6666 in the human Apolipoprotein B transcript. APOBEC1 has been linked to cancer development in mice and rabbits. Its oncogenic mechanisms have been ascribed to its ability to target RNA, but recent observations show that APOBEC1 can induce a mutator phenotype in mammalian cells., To understand whether the oncogenic potential of APOBEC1 is mediated by DNA or RNA editing, we present a bacterial screen to isolate RNA-editing defective mutants of APOBEC1 proficient in DNA editing., We have characterized the selected mutants for their ability to edit the physiological transcript - through an assay that visualizes RNA editing in live cells - and for their ability to induce a mutator phenotype – through their ability to restrict lentiviral replication or through a mutation assay. All selected mutants lost their ability to edit mRNA, however some of them are able to induce tumorigenic features in human cells (transformation assay, soft-agar). This suggests that the ability to target DNA plays a central role in the oncogenic potential of APOBEC1.

P22.7
In vitro risk assessment of pure *trans*-anethole and of a herbal extract from *Foeniculum vulgare* (fennel), containing *trans*-anethole

S. Vannini[1,*], S. De Marco[1,2,*], S. Levorato[1], C. Zadra[2], M. Moretti[1], M. Villarini[1]
*Department of Pharmaceutical Sciences,University of Perugia: [1]Unit of Public Health, and [2]Unit of Food and Nutritional Science, *These authors contributed equally to this work.*

Herbal products such as herbal teas and plant food supplements (PFS) are widely used in Western diets. Although many consumers equal 'natural' with 'safe', botanical preparations may contain ingredients known to be toxic and even genotoxic or carcinogenic.One of the categories of plant metabolites of concern are the alkenylbenzenes, flavourings regulated by EU Regulation 1334/2008, used to improve or modify the odour and/or taste of several foods.*trans*-Anethole is a non-EU regulated flavouring substance present in the essential oils of a variety of plants, notably *Foeniculum vulgare* (fennel), *Magnoliaceae* (star anise) and *Pimpinella anisum L.*(anise).This study describes the *in vitro* cytotoxic, genotoxic, and apoptotic activities of *trans*-anethole in the HepG2 cell line. The HepG2 cell line was treated both with pure *trans*-anethole and with the extract of camomile and fennel infusion.To achieve this goal, *trans*-anethole in a food matrix was determined by GC-MS and double-staining (acridine orange and DAPI) viability assay, single-cell microgel-electrophoresis (comet) assay, mitochondrial membrane potential (Δψm) assay and DNA fragmentation analyses were conducted.

P22.8
The MAPEC_LIFE study (LIFE12 ENV/IT/000614): monitoring air pollution effects in children for supporting public health policy

M. Verani[1], A. Carducci[1], B. Casini[2], M.R. Marrese[3], A. Bonetti[4], S. Monarca[5], M.A. De Donno[6], E. Carraro[7], U. Gelatti[8], the MAPEC_LIFE Study Group
[1]Department of Biology, University of Pisa, Pisa, Italy, [2]Department of Translational Research, N.T.M.S., University of Pisa, Pisa, Italy, [3]Comune di Brescia, Brescia, Italy, [4]Centro Servizi Multisettoriale e Tecnologico – CSMT Gestione S.c.a.r.l., Brescia, Italy, [5]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy , [6]Department of Biological and Environmental Sciences and Technology, University of Salento, Lecce, Italy, [7]Department of Public Health and Pediatrics, Torino, Italy, [8]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy

The use of genetic biomarkers has been studied largely in adult population exposed to air pollution, but few studies have investigated genetic damage in children. The aim of the project is to evaluate the associations between the concentration of urban air pollutants and early biological effects in children. The study will be carried out on 1000 children 6-8 years old living in five Italian towns in two different seasons by analyzing two biomarkers of early biological effects: DNA damage detected with the comet assay and frequency of micronuclei in buccal cells. A questionnaire will be used to collect the details of children diseases, socio-economic status, exposures to other pollutants and life-style. Ultra-fine particulate samples (PM 0.5) collected in the school areas will be analyzed for PAHs and nitro-PAHs concentrations, lung toxicity and in vitro genotoxicity on bacterial and human cells. All data will be statistically tested to investigate the possible associations between levels of air pollutants, air mutagenicity and early effect biomarkers. The final purpose of the project will be to elaborate a model for calculating the global absolute risk of early biological effects.

P22.9
Genotoxicity detection and flow cytometric evaluation of TiO_2 nanoparticles in human PBMC

C. Andreoli[1], G. Leter[2], B. De Berardis[1], P. Degan[3], I. De Angelis[1], F. Pacchierotti[2], R. Crebelli[1], F. Barone[1], A. Zijno[1]
[1]Dept Environment and Primary Prevention, Istituto Superiore di Sanità, Roma, [2]Laboratory of Toxicology, ENEA Casaccia, Rome, [3]Molecular Mutagenesis and DNA Repair, Istituto Nazionale per la Ricerca sul Cancro, Genova Italy

Nano and microparticles of TiO_2, characterized by TEM and SEM, were tested in PBMC for capability to interact with DNA. Single and double strand breaks, 8-oxodG and micronucleus levels were measured. Furthermore, ability of different forms of $TiO_2$to cross cell membranes as well as ROS production and cell viability.was investigated at 0° and 37°C by flow cytometry (FCM). For Comet assay and 8-oxodG induction, cells were treated for 6 or 24hs (10-200 μg/ml). Results indicated a slight increase of DNA damage and a significant dose-related increase in oxidated Guanine. The InVitMn assay was performed on proliferating lymphocytes and Cyt B was added simultaneously or subsequently to treatments (50-200 μg/ml). Marginal and not reproducible increases of Mn were observed only for microparticles. FCM SSC data show that only monocytes are prone to take up TiO_2 particles in dose-dependent trends at 37°C. Lower uptake was observed at 0°C. The results of cell viability and ROS production agreed with those from uptake. These results suggest that all forms of TiO_2 were able to induce oxidative damage in PBMC independently by their size. That damage was not converted in frank chromosome breaks in lymphocytes possibly because it was mainly induced on monocytes or repaired in lymphocytes after stimulation and reactivation of cell cycle.

O22.1
The RNA editing enzyme APOBEC1 induces somatic mutations and its mutational signature is present in esophageal adenocarcinomas

G. Saraconi, F. Severi, C. Sala, G. Mattiuz, S.G. Conticello
Core Research Laboratory, Istituto Toscano Tumori - AOU Careggi, Firenze, Italy

The AID/APOBECs are deaminases that act on cytosines in a diverse set of pathways, and some of them have been linked to the onset of genetic alterations in cancer. Among them, APOBEC1 is the only family member to physiologically target RNA, as the catalytic subunit in the

Apolipoprotein B mRNA editing complex. APOBEC1 has been linked to cancer development in mice but its oncogenic mechanisms are not yet well understood., Here we show that expression of APOBEC1 induces a mutator phenotype in vertebrate cells, likely through direct targeting of genomic DNA. Moreover, we find the presence of an AID/APOBEC mutational signature, which mimics the one preferred by APOBEC1 *in vitro*, in esophageal adenocarcinomas, a type of tumor where APOBEC1 is expressed., Our findings suggest that the ability of APOBEC1 to trigger genetic alterations represents a major layer in its oncogenic potential. Such APOBEC1-induced mutator phenotype could play a role in the onset of esophageal adenocarcinomas. APOBEC1 could be involved in cancer promotion since the very early stages of carcinogenesis, as it is highly expressed in Barrett's esophagus, a condition often associated to esophageal adenocarcinoma.

O22.2
Investigation of the cytotoxic, genotoxic and apoptotic activities of *trans*-cinnamaldehyde: pure compound and botanical matrix (cinnamon tea)

S. De Marco[1,2], S. Levorato[1], S. Vannini[1], C. Zadra[2], M. Moretti[1], M. Villarini[1]

[1]Unit of Public Health, Dept of Pharmaceutical Sciences, Univ. of Perugia, Perugia, Italy, [2]Unit of Food and Nutritional Science, Dept of Pharmaceutical Sciences, Univ. of Perugia, Peruugia, Italy

Present-day society is increasingly demanding food products made of natural ingredients. While many consumers equal 'natural' with 'safe', botanical preparations may contain ingredients known to be genotoxic or carcinogenic., Alkenylbenzenes, flavourings regulated by EU Reg. 1334/2008, are one of the categories of concern., *trans*-Cinnamaldehyde, a non-EU regulated alkenylbenzenic compound, is a constituent of the essential oils of *Cinnamomum osmophloeum*(cinnamon)., The present study describes the determination by GC-MS of *trans*-cinnemaldehyde in a food matrix and the *in vitro*cytotoxic, genotoxic and apoptotic activities in the HepG2 cell line., Double-staining (acridine orange and DAPI) viability assay, comet assay, mitochondrial membrane potential ($\Delta\psi$m) assay and DNA fragmentation analysis were conducted., The HepG2 cell line was treated both with pure *trans*-cinnamaldehyde and with the extract of orange&cinnamon tea., The results of HepG2 cells treated with *trans*-cinnamaldehyde for 4 h showed a significant increase of primary DNA damage. Loss of the mitochondrial membrane potential, an early apoptosis marker, and DNA fragmentation were observed, respectively, after 4h and 24h of exposion.

O22.3
Genetic and environmental influence on the activity of the DNA repair enzyme OGG1: a twin study

L. Nisticò[1], F. Mazzei[2]; E. Medda[1], A. Minoprio[1], V. Simonelli[2], V. Toccaceli[1], B. Bocca[2], A. Alimonti[2], M.A. Stazi[1], E. Dogliotti[2]

[1]National Center of Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità, Rome, Italy, [2]Department of Environment and Primary Prevention, Istituto Superiore di Sanità, Rome, Italy

The activity of the main enzyme of oxidative DNA damage repair, OGG1, was measured on lymphocyte protein extracts of 106 Italian twins (37 monozygotic (MZ), 16 dizygotic (DZ) pairs) by an in vitro cleavage assay. The contributions of genetic and environmental effects were assessed using standard univariate twin modeling based on linear structural equations., OGG1activity was highly correlated both in MZ (r=0.77) and in DZ (r=0.66) pairs, suggesting that shared (C) and unshared (E) environmental factors substantially contributed to total variance (best model: C=72%; 95%CI:57-83, E=28%; 95%CI:17-43). Birth weight correlated with OGG1 activity (N=97; coefficient=-0.0002; $p<0.001$) and, when introduced as a covariate in the model, markedly reduced C estimate (36%; 95%CI:0-79). We also found that OGG1 activity was inversely correlated with blood levels of manganese (Coefficient=-0.03; p=0.008) and cadmium (Coefficient=-0.16; p=0.009). Alterations in the antioxidant defense system have been reported as a signature of premature birth. Our study suggests that the activity of OGG1 is part of this signature. Moreover, environmental contaminants are important modulators of this activity.

O22.4
Potential use of the isoflavonic phytoestrogen genistein in thyroid cancer therapy

G. Frenzilli, P. Guidi, M. Bernardeschi, V. Scarcelli, P. Lucchesi, C. Ghezzani, S.M. Ferraris, P. Fallahi, A. Antonelli

Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

Isoflavone intake was found to be related to thyroid dysfunction since 1930s after observing that goiters occurred in rats consuming soy. Recently, it has been shown that genistein, a naturally occurring isoflavonic phytoestrogen, displayed growth inhibition in thyroid cancer cells. *In vitro* and *in vivo* studies also reported ginestein as a DNA damage protector, acting by interfering in ROS accumulation. These findings suggest that genistein may provide a useful therapeutic intervention in thyroid cancer therapy. We planned to evaluate if genistein is able to exert anti-neoplastic action in primary human papillary thyroid cancer cells (PTC). Thyroid tissues obtained from patients and control samples of normal thyroid tissue were treated with genistein (1-10-50-100 μM). A co-treatment with bleomycin was also performed. Cell viability (MTT assay) and proliferation assay were carried out. DNA primary damage was evaluated at 4 and 24 h by the Comet assay, nuclear abnormalities and MN frequency by the Cytome assay. Preliminary data suggest that genistein exerts antineoplastic action in primary thyrocytes from PTC, reduces cell viability of PTC cells, while not inducing genotoxic effects.

O22.5
A panel of tests to evaluate genotoxicity and carcinogenicity induced *in vitro* by metal oxide nanoparticles

C. Uboldi[1], S. Di Bucchianico[1], T. Brunetti[1], S. Luongo[1], F. Coppedè[1], C. Delpivo[2], A. Costa[2], L. Migliore[1]

[1]Medical Genetics Unit – Dept. of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy, [2]Institute of Science and Technology for Ceramics, National Research Council of Italy, Faenza, Italy

To investigate the genotoxicity and the carcinogenicity of nanomaterials, we propose a panel of assays including the cytokinesis-block micronucleus cytome assay (CBMN-cyt) and the cell transformation assay (CTA) in Balb/3T3 mouse fibroblasts. We applied the tests to study zirconium (ZrO_2 NP) and titanium dioxide (TiO_2 NP) nanoparticles. By CBMN-cyt the evaluation of the replication index and of apoptotic/necrotic events indicated that both NP induced cytostasis and cytotoxicity, while the assessment of the genotoxic potential produced different findings. In fact, ZrO_2 NP but not TiO_2 NP increased the frequency of micronuclei, nucleoplasmic bridges and nuclear buds. Accordingly, CTA showed that ZrO_2 NP had carcinogenic potential since they have induced the formation of type-III foci in Balb/3T3 cells *in vitro*. Moreover, the coating of ZrO_2 NP and TiO_2 NP with silica and with sodium citrate confirmed that the functionalization plays a pivotal role in the toxic mechanisms of NP: the presence of silica slightly but not significantly reduced the genotoxicity of ZrO_2 NP and TiO_2 NP, whilst the carcinogenic potential and the cytotoxic effects were enhanced in the presence of citrate.

23 - Transcription Mechanisms and Networks

P23.1
454 pyrosequencing and *de novo* assembly of Antarctic krill (*Euphausia superba*) transcriptome

A. Biscontin[2], P. Martini[2], C. De Pittà[2], C. Romualdi[2], M. Teschke[1], S. Frickenhaus[3,4], L. Harms[3], U. Freier[5], S. Spahic[1], S. Jarman[6], S. Kawaguchi[6] , R. Costa[2], B. Meyer[1]
[1]Alfred Wegener Institute, Helmholtz Centre for Polar and marine Research, Section Polar Biological Oceanography, Bremerhaven, Germany, [2]Dipartimento di Biologia, Università degli Studi di Padova, Padova, Italy, [3]Alfred Wegener Institute, Helmholtz Centre for Polar and marine Research, Section Scientific Computing, Bremerhaven, Germany, [4]Hochschule Bremerhaven, An der Karlstadt 8, 27568 Bremerhaven, Germany, [5]SC-Scientific Consulting, Münchener Str. 41a, D-41472 Neuss, Germany, [6]Australian Antarctic Division, Kingston Tasmania, 7050, Australia

The Antarctic krill, *Euphausia superba*, has a key position in the Southern Ocean food web. It serves as direct link between primary producers and apex predators. The southwest Atlantic sector of the Southern Ocean experiences one of the most profound environmental changes worldwide. Up to now, we have only cursory information about krill's genomic plasticity to cope with the ongoing environmental changes such as seawater temperature rise and ocean acidification. Here, we present two cDNA normalized libraries from whole krill and krill heads sampled at different seasons (BM libraries), that were combined with two datasets already published, in order to produce the first krill master transcriptome. Thanks to BM libraries, we introduce 25% of new krill transcripts in the master transcriptome that now includes nearly all the enzymes involved in the primary oxidative metabolism as well as all genes involved in glycogenesis, glycogen breakdown, gluconeogenesis, fatty acid synthesis and β-oxidation. With these features, the master transcriptome provides the most update picture of metabolic pathways in krill and will provide a major resource for future physiological and molecular studies.

P23.2
Tab2 controls ERα complexes and resistance to endocrine therapy

S. Reineri[1,2], L. Ricci[1,2], V. Miano[1,2], A. Lykkesfeldt[3], S. Cutrupi[1], M. De Bortoli[1,2]
[1]Center for Molecular Systems Biology, Univ. Turin, Italy, [2]Dept Clinical & Biological Sciences, Univ. Turin, Italy, [3]Dept. of Breast Cancer Research, Inst. Cancer Biology, Copenhagen, Denmark

Tab2 mediates response to different transduction pathways by dismissing NCoR corepressor from gene regulatory regions, thus leading to gene derepressione in several contexts. We demonstrated that either Tab2 knockdown or a peptide mimicking the ERα interacting motif restore the antiproliferative response to tamoxifen in spontaneously tamoxifen-resistant MCF7-TAMR breast cancer cells (Cutrupi, 2012). We identified the Tab2 domain that interacts with ERα and, by narrowing down the essential interaction motif, we found that a 15-aa peptide competed for ERα/Tab2 interaction, in vitro. Next, this peptide was conjugated with the Tat minimal carrier domain. Treatment of MCF7-TAMR cells with the Tat-Tab2 peptide, but not with a scrambled one, resulted in marked decreased proliferation in response to tamoxifen. The activity of Tat-Tab2 was confirmed also by expressing the entire Tab2 (406-531) domain in these cells. Since Tab2 interacts with other steroid receptors, as AR and PgR, or with the protein Bcl3, all having homology to ERα (1-45), our results may have relevance in other contexts, like in prostate or ovarian cancers, in Alzheimer's disease or in neuronal differentiation.

P23.3
The Cop9 signalosome is involved in the regulation of lipid metabolism and of transition metals uptake in *S. cerevisiae*

A. Cirigliano[1], V. Licursi[1], T. Rinaldi[1], B. Mattei[1], G. Serino[1], V. Vapore[1], J. Z. Zimbler[2], S. Tabash[2], E.Pick[2], R. Negri[1]
[1]Istituto Pasteur - Fondazione Cenci Bolognetti, Dipartimento di Biologia e Biotecnologie, Sapienza Università di Roma, [2]Department of Biology and Environment, University of Haifa, Tivon, Israel

The COP9 signalosome (CSN) is a highly conserved eukaryotic protein complex which regulates the Cullin-RING family of ubiquitin ligases and carries out a deneddylase activity that resides in subunit 5 (CSN5*)*. Whereas CSN activity is essential for development of higher eukaryotes, several unicellular fungi, including the budding yeast *S. cerevisiae* can survive without a functional CSN. Nevertheless, the budding yeast CSN is biochemically active and deletion mutants of each of its subunits exhibit deficiency in deneddylation of cullins. To further characterize CSN function in *S. cerevisiae*, we performed a transcriptomic and proteomic analysis of a strain deleted in *CSN5/RRI1*gene, coding for the only canonical subunit of the complex. We show that Csn5 is involved in the modulation of the genes controlling aminoacid and lipid metabolism, and especially ergosterol biosynthesis. These alterations in gene expression correlate with specific phenotypes such as reduced ergosterol levels and Heme content and increased intracellular zinc content which we observed in *csn5* null mutant cells. We show that some of these regulatory effects of Csn5 are conserved through evolution, since they were previously observed in other eukaryotic organisms such as *A. nidulans, A. thaliana* and *D. melanogaster*. Our results suggest that the diverged budding yeast CSN is more conserved than was previously thought.

P23.4
Functional studies of Mesorhizobium loti proteins

A. Rivellino, I. Baglivo, L. De Cesare, S. Esposito, M.M. Marino, L. Muscariello, P.V. Pedone
Di.S.T.A.Bi.F., Second Unversity of Naples, Caserta, Italy

Mesorhizobium loti is a Gram negative bacterium member of Rhizobia. Analyzing protein data banks, we found in *M. loti* ten homologues of Ros protein from *A.tumefaciens* [2].We studied the DNA binding ability of five of the ten homologues founded in *M. loti*, showing that they can bind the same DNA target sequence of Ros, even presenting some differences in the zinc coordinating positions respect to Ros[3].We also demonstrated how other chemical interactions surrogate the zinc coordination role to mantein the correct and functional fold in Ml4 and Ml5 [4]. The five protein are homologues of the MucR protein from *Sinorhizobium meliloti*. This protein is an expression regulator of exopolysaccharides involved in biofilm formation. We demonstrate that the five proteins recognize a DNA target sequence present in the promoter of the *exoY*from *M. loti.* Here we analyze the expression of all proteins during planktonic growth and during the formation of biofilm. Our preliminary results assess that just three of the five proteins are expressed in planktonic conditions, while none of the five proteins of *M.loti* is expressed in biofilm. Our next aim is to correlate the expression of proteins to the *exoY* gene to demonstrate their gene expression regulatory function.

O23.1
DNMT3s and miRs-29 in EMT/MET dynamics: a role for HNF4α

C. Battistelli[1], V. de Nonno[1], A. M. Cozzolino[3], S. A. Ciafrè[2], L. Amicone[1], M. Tripodi[1,3], C. Cicchini[1]
[1]Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Cellular Biotechnologies and Haematology, Section of Molecular Genetics, "Sapienza" University of Rome, Rome, Italy, [2]Department of Biomedicine and Prevention; University of Rome "Tor Vergata", Rome, Italy, [3]National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy

Epithelial-to-Mesenchymal Transition (EMT) and the reverse Mesenchymal-to-Epithelial Transition (MET) are manifestations of cellular plasticity that imply a dynamic and profound gene expression reprogramming. While a major epigenetic code controlling the

coordinated regulation of a whole transcriptional profile is guaranteed by DNA methylation, DNA methyltranseferases activities in EMT/MET dynamics is still largely unexplored. ,We investigated molecular mechanisms directly linking the master effector of MET HNF4α to the*de novo* DNA methyltransferases DNMT3A and DNMT3B. HNF4α silencing in differentiated hepatocytes was found sufficient to induce both DNMT3A and DNMT3B positive modulation. Moreover, evidence have been gathered for (i) the inverse correlation between the levels of DNMT3A and DNMT3B and the expression of miR-29a and miR-29b, and (ii) the role of HNF4α as direct regulator of miR-29a-b transcription. Notably, during the TGFβ-induced EMT, DNMT3s pivotal function has been proved. Overall, HNF4α functional activities in maintaining hepatocyte identity include the control of epigenetic modifications featured by DNMT3A and DNMT3B this occurring through the direct regulation of miR-29a and 29b expression.

O23.2
A novel regulatory switch controls the expression of *Neisseria meningitidis*NHBA at physiologically relevant temperatures

S. Borghi[1], A.F. Haag[2], A. Antunes[2], V. Scarlato[1], I. Delany[2]
[1]Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Bologna, Italy, [2]Novartis Vaccines, Microbial Molecular Biology, 53100 Siena, Italy

*Neisseria meningitidis*colonizes the nasopharynx of humans and pathogenic strains can disseminate into the bloodstream causing septicemia and meningitis. Neisserial Heparin Binding Antigen (NHBA), also known as GNA2132, is part of a multicomponent vaccine against *Nm* serogroup B, Bexsero™., NHBA expression is increased at 30 and 32°C, a range which reflects its nasopharyngeal niche. We determined that temperature-controlled expression of NHBA was conserved among all *Nm* tested. While only minor effects of temperature on transcript levels were measurable by microarray and RT-PCR analysis, substitution of the regulatory regions upstream of the GNA2132 coding sequence abrogated temperature-dependent control of NHBA expression, suggesting that the regulation is not due to protein stability but likely to be governed by a post-transcriptional mechanism. Site-specific mutational analysis of the promoter elements, 5'UTR and features of the coding sequence have revealed key nucleotides important for regulation of expression of NHBA. A model of our understanding of the molecular mechanism controlling the thermoregulation of NHBA will be discussed.

O23.3
Multicellularity in yeast: heat shock proteins as actors of an epigenetic regulatory network

V. Cappelletti[1,4], I. Stefanini[1], L. Berná[2], M. Ramazzotti[3], A. Cestaro[1], C. Romualdi[4], M. Kapushesky[5], I. Gut[6], A. Csikász-Nagy[1,7], D. Cavalieri[1]
[1]Department of Computational Biology, Research and Innovation Centre, Fondazione Edmund Mach, Trento, IT, [2]Unidad de Biología Molecular, Institut Pasteur Montevideo, Mataojo 2020, Montevideo, Uruguay, [3]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, IT, [4]Department of Biology, University of Padua, IT, [5]EMBL Outstation-Hinxton, European Bioinformatics Institute, Cambridge, UK, [6]Centro Nacional de Análisis Genómico, Barcelona, SP, [7]King's College London, UK

Colony formation is a fascinating trait described in unicellular organisms as a possible step towards multicellularity. *Saccharomyces cerevisiae* has been shown to be a powerful model to deepen into this evolutionary process thanks to its ability to grow in multicellular structures in response to environmental changes. We focused on the filamentous phenotype observed in the M28 natural *S. cerevisiae*strain and recently associated to prion response. M28 sporal derivatives, showing a 2:2 Mendelian segregation of multicellular phenotype, represent a unique model to understand the inheritance of the multicellular behaviour. Remarkably, we observed the ability of M28 meiotic segregants to switch from filigreed to smooth phenotype and vice-versa, with a reversion rate that is perturbed by prion removing agents, suggests an epigenetic control of this mechanism. In order to identify a gene expression profile associated to this phenotypic transition we performed a time course microarray transcriptional analysis followed by network analysis. Furthermore whole genome comparative analysis on 12 sporal derivatives provide further insight in how the genotype is translated into the phenotype.

O23.4
Impairment of the COP9 signalosome in yeast leads to activation of autophagy

A. Cirigliano[1,2], R. Santomartino[2], A. Stirpe[2], L. Bramasole[3], D. Dell'Edera[1], G. Serino[2], E. Pick[3], R. Negri[2], T. Rinaldi[2]
[1]Associazione Gian Franco Lupo, Azienda Sanitaria Locale di Matera, Italy, [2]Pasteur Institute-Cenci Bolognetti Foundation, Dept of Biology and Biotechnology "Charles Darwin", Univ. of Rome La Sapienza, Rome, Italy, [3]Dept of Biology & Environment, Univ. of Haifa, Israel

The COP9 signalosome (CSN) complex is a conserved multi-subunit enzyme that shows a high similarity with the proteasome lid subcomplex. The intact CSN complex harbors a metalloprotease domain, ascribed to its catalytic subunit Csn5, and it is required for hydrolysis of related-ubiquitin1 Rub1/Nedd8 (i.e. deneddylation) from the cullin scaffold subunit of cullin-RING ubiquitin-E3s. CSN activity regulates ubiquitination of more than 20% of cellular ubiquitinated proteins in human and this complex is conserved in evolution. Here we show that, in S.cerevisiae, a CSN5 null mutant exhibits an enlarged vacuole, and an array of related metabolic phenotypes. We further show that lack of Csn5 leads to autophagy and mitochondrial dysfunction. In addition, knocking out CSN5 function in a proteasome dysfunction mutant results in a semilethal phenotype, implying that the proteasomal function is necessary in the absence of the CSN.

O23.5
The Dof protein DAG2 is a positive regulator of the phyB-mediated seed germination process

S. Santopolo, E. Minutello, A. Boccaccini, D. Capauto, R. Lorrai, V. Ruta, P. Costantino, P. Vittorioso
Istituto Pasteur Fondazione Cenci Bolognetti, Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma, Rome, Italy

Germination of Arabidopsis seeds needs Red light and is mediated mainly by the photoreceptor phyB. Red light modulates the levels of gibberellins (GA), the phytohormone inducing germination. The TFs PIL5 and DAG1 represent two key repressors of the molecular pathway leading to seed germination. DAG1 negatively regulates GA biosynthesis by directly repressing the GA biosynthetic gene *AtGA3ox1*. The Dof proteins DAG1 and DAG2 share a high degree of aminoacid identity. Interestingly, *DAG1* inactivation considerably increases the germination capability of seeds, while *dag2* mutation results in seeds with a substantially lower germination potential, indicating that these factors might have opposite roles., Recently, we studied *DAG2* expression in seeds under different experimental conditions. These data showed that *DAG2* is positively regulated by environmental factors triggering germination (light, imbibition), whereas its expression is reduced by PIL5 and DAG1, repressors of this process. Consistently, germination of *dag2* mutant seeds under light is significatively reduced, proving that DAG2 is a positive regulator of the seed germination light-mediated process.

24 - Noncoding RNA

P24.1
Post-transcriptional regulation of *PHOX2B* gene expression

T. Bachetti[1], E.Di Zanni[1], R. Ravazzolo[1,2], I. Ceccherini[1]
[1]Medical Genetics Unit, Istituto Giannina Gaslini, Genova, Italy, [2]Department of Neurosciences, Rehabilitation, Ophthalmogy, Genetics, Maternal and Child Health and CEBR, Università degli Studi di Genova, Genova, Italy

Neuroblastoma (NB) is one of the most frequent and severe solid tumors in childhood., As the *PHOX2B* gene is overexpressed in both NB samples and cell lines, the regulation of *PHOX2B*expression mediated by upstream and/or downstream regions of the gene may be considered a novel pharmaceutical target in NB., In particular, in this work, we report *in silico* and *in vitro* characterization of the *PHOX2B* 3' untranslated region (3'UTR)., The search for elements known to regulate the mRNA stability could identify three AU-rich elements (AREs) in the more distal region of the 3'UTR, in addition to several sites putative for miRNAs binding. Combined analysis between results obtained by *in silico* prediction of regulatory elements and results obtained by analysis of phylogenic conservation of the 3'UTR allowed us to better define the regions likely responsible for PHOX2B mRNA regulation., The cloning of the entire 3'UTR *PHOX2B*downstream the Luciferase gene allowed us to confirm the role of the 3'UTR in decreasing mRNA stability and analysis of databases for miRNA expression profiles and miRNA sites predicted on the 3'UTR allowed us to identify miRNA responssible for *PHOX2B* overexpression in NB.

P24.2
miR-17-92 counterbalances MYC and fine-tunes MYC-centred regulatory networks in lymphoma maintenance

M. Mihailovich[1], M. Bremang[1], E. Vitale[1], G. Varano[2], F. Zambelli[3], G. Pavesi[3], S. Casola[2], T. Bonaldi[1]
[1]Department of Experimental Oncology, European Institute of Oncology, Milan 20139, Italy, [2]IFOM, FIRC Institute of Molecular Oncology Foundation, Milan 20139, Italy, [3]Department of Biosciences, Milan University, Milan 20133, Italy

The synergism between MYC and miR-17-19b has been demonstrated in tumor initiation; however little is known about their functional interplay in full-blown lymphoma. In this study, we investigated the role of miR-17-19b in a model of MYC-driven B cell lymphoma through a systems biology approach that combines quantitative proteomics with transcriptomics, 3'UTR analysis and *in vivo* phenotypization. We identified of more than a hundred novel miR-17-19b targets, out of which 40% are shared with MYC, indicating the role of these miRNAs in fine-tuning MYC transcriptional activity. We also observed that miR-17-19b negatively regulates translation of MYC through the novel target, Chek2. Modulation of Chek2-dependent HuR phosphorylation increases its binding to *MYC* mRNA. HuR recruits RISC, thus inhibits MYC translation. In line with these data, a mild increase in miR-17-19b reduces tumor aggressiveness, both *in vitro* and *in vivo*., Our data show that the functional interplay between MYC and miR-17-19b is plastic and changes dynamically during tumor development, as a consequence of the transcriptome plasticity. In particular, the distinct outcomes of miR activity result from an altered pattern of miRNA-targets, due to extensive 3' UTR shortening. Hence, while miR-17-92 potentiates MYC pro-tumorigenic function during lymphomagenesis, it dampens MYC oncogenic activity in established tumors by dempening MYC expression and functions.

P24.3
A common polymorphism within MSLN affects miR-611 binding site and is associated with serum mesothelin (SMRP) in healthy people

C. De Santi[1], S. Garritano[2], R. Silvestri[1], O. Melaiu[1], M. Cipollini[1], E. Barone[1], M. Lucchi[3], R. Barale[1], L. Mutti[4], F. Gemignani[1], A. Bonotti[5], R. Foddis[5], A. Cristaudo[5], S. Landi[1]
[1]Department of Biology, University of Pisa, Via Derna, 1, 56126 Pisa, Italy, [2]Laboratory of Computational Oncology, Centre for Integrative Biology, CIBIO, University of Trento, 30123 Trento, Italy, [3]Division of Thoracic Surgery, Cardiac and Thoracic Department, University of Pisa, Via Paradisa 2, Pisa 56124, Italy, [4]Department of Medicine, Vercelli Teaching Hospital, 13100 Vercelli, Italy, [5]Section of Occupational Medicine, School of Medicine and Surgery, University of Pisa, 56124 Pisa, Italy

Soluble Mesothelin Related Peptide (SMRP) has been proposed as diagnostic marker for Malignant Pleural Mesothelioma (MPM). In a previous study we found an association between rs1057147 G>A within 3'UTR of MSLN gene and SMRP levels. In this study, the association analysis was verified in 759 subjects. SMRP performance as diagnostic biomarker improved by considering the genotype rs1057147 in ROC curves analysis. The polymorphism was evaluated in in vitro experiments as putative miRSNP (i.e. SNP affecting miRNA binding sites). Dual luciferase assays on Met-5A cells showed a significantly lower reporter activity when the vector harboured the G-allele as compared to A-allele. In silico predictions highlighted miR-611 and miR-887 as candidate microRNAs involved in this process. In co-trasfection assays, miR-887 mimic caused a reduced reporter activity of vectors harboring A or G alleles, while miR-611 was effective only on the vector harboring the G allele. Transfection of these miRNAs into Mero-14 cells significantly reduced endogenous MSLN protein. Altogether, these experiments supported the notion that rs1057147 could act as miRSNP and most likely affects a binding site for miR-611.

P24.4
Identification of sRNA networks and their role in regulating virulence of *Neisseria meningitidis*

G. Golfieri[1,2], L. Fagnocchi[1,2], R. D'Aurizio[1], G. Torricelli[1], S. Guidotti[1], N. Pacchiani[1], S. Censini[1], V. Scarlato[2], I. Delany[1]
[1]Novartis Vaccines, Siena, Italy, [2]University of Bologna, Bologna, Italy

Neisseria meningitidis (Nm), the leading cause of bacterial meningitis, can adapt to different host niches during human infection. Small non-coding RNAs (sRNAs) have been identified as crucial regulatory elements for bacterial stress responses and virulence, but only few sRNAs have been identified and characterized to date in Nm., Pathogens coordinate important responses in gene expression to iron availability. We investigated the Nm transcriptional landscape using RNAseq, by analyzing total RNAs extracted from Nm cultured under standard or iron limiting *in vitro* growth conditions. Manual curation of the RNAseq data detected the expected expression profiles of known structural and regulatory sRNAs (such as 4.5S, 6S, NrrF and AniS), confirming the power of our RNAseq in identifying sRNAs. This analysis generated a list of 39 novel sRNAs, 11 of which were validated by Northern blotting and/or primer extension. Finally, we describe the characterization of a novel sRNA unique to Nm, which could be involved in the regulation of genes relevant for the intracellular survival of pathogenic *Neisseriae*. These findings could help unravel the mechanisms of Nm adaptation to the host environment.

P24.5
miRVine: a microRNA expression atlas of grapevine based on small RNA library sequencing

J. Belli Kullan[1], D. Lopes Paim Pinto[1], E. Bertolini[1], M. Fasoli[2], S. Zenoni[2], M. Pezzotti[2], B. Meyers[3], L. Farina[4], M.E. Pè[1], E. Mica[1,5]
[1]Inst. Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy, [2]Dept. Biotechnology, Univ. of Verona, Verona, Italy, [3]Dept. Plant and Soil Sciences, Univ. of Delaware, Newark, USA, [4]Dept. Computer, Control and Management Engineering, Sapienza Univ., Rome, Italy, [5]Genomics Research Centre, Consiglio per la Ricerca e la Sperimentazione in

Agricoltura, Fiorenzuola d'Arda (PC), Italy

Genetic and epigenetic regulations, mostly driven by small non-coding RNAs, play a crucial role to define genetic programming in plant biology and development. Focusing on miRNAs, we present the first comprehensive expression atlas in *Vitis vinifera*., We analyzed 68 small RNA libraries, which were prepared from 12 organs harvested on different developmental stages. We identified 110 known and annotated 137 novel miRNAs, some of which belonging to known families and others completely new., Our expression atlas gives a clear picture of miRNA regulation and homeostasis in the whole plant during its lifecycle. Very few miRNAs may be defined as tissue specific, however 22 miRNAs have been identified as stamen specific. Most of the miRNAs show low expression levels, whereas 32 are present in all tissues and are mainly highly expressed. In each organ the developmental stages share 30—70% of miRNAs and their modulation leads to the regulation of plant development. Targets predictions enrich our analysis and suggest a first functional description of hundreds of miRNAs. Our findings represent the most complete expression atlas for woody species, paving the ground for future functional studies.

O24.1
A novel long noncoding RNA promoting neural induction of human induced pluripotent stem cells

V. Bevilacqua[1], U. Gioia[1,4], J. Lenzi[2], A.F. Tortorelli[1], V. Di Carlo[1], P. Laneve[2], I. Bozzoni[1,2], A. Rosa[1], E. Caffarelli[2,3]

[1]Dept Biology and Biotechnology, Sapienza University of Rome, Italy, [2]Center for Life Nano Science, Istituto Italiano di Tecnologia, Sapienza University of Rome, Italy, [3]Institute of Biology, Molecular Medicine and Nanobiotechnology-CNR, Rome, Italy, [4]Current address:IFOM Foundation - The FIRC Institute of Molecular Oncology Foundation, Milan, Italy

A comprehensive analysis of the mammalian transcriptome has revealed that long noncoding RNAs (lncRNAs) account for a large fraction of cellular transcripts. Although their functional importance has been underestimated for a long time, lncRNAs are now considered crucial gene regulators in the cell differentiation process. Interestingly, it has been shown that a half of all human lncRNAs is expressed in the nervous system, even if the function of the vast majority of neuronal-expressed lncRNAs is still unknown., Using human neuroblastoma-derived cells, and human induced Pluripotent Stem Cells (iPSCs) as model systems to recapitulate *in vitro* neuronal differentiation, we identified a novel neural lncRNA, linc-NeD125, as the host gene for miR-125b-1, a microRNA with a well-established role in neuronal differentiation. We focused on linc-NeD125 gene structure and expression and discovered that specific transcriptional and post-transcriptional mechanisms contribute to the coordinated biogenesis of linc-NeD125 and miR-125b-1. Finally, we investigated linc-NeD125 function and found that this lncRNA acts as key player in neural lineage commitment, independently from the hosted miRNA.

O24.2
MiR-21 over-expression in K562 cells silenced for ferritin heavy chain (FHC) is mediated by oxidative stress

F. Biamonte, F. Zolea, I. Aversa, M. Panebianco, F. Trecroci, G. Cuda, F.S. Costanzo

Dept Experimental and Clinical Medicine, Magna Græcia University of Catanzaro

The oncomiR-21, often over-expressed in several types of malignancies, is involved in the control of cell proliferation, apoptosis, invasion and metastasis. Moreover, its expression is elicited in response to oxidative stress. The iron-binding protein ferritin, and particularly its heavy subunit (FHC), plays a key role in redox homeostasis. We analyzed the expression of miR-21 in wild-type and FHC-silenced (shFHC) K562 cells. FHC silencing induced an increase of about 2-fold in ROS levels and it is accompanied by a significant up-regulation (10-fold) of miR-21 ($P< 0.001$). Treatment of shFHC K562 with the ROS inhibitor NAC (N-acetyl-L-cysteine) brought miR-21 levels back to that of wild-type cells. We further determined the protein expression of three experimentally validated miR21 targets (p53, MLH1 and MSH2). Our results show that the up-regulation of miR-21 is strongly associated with a decreased expression of the predicted targeted gene products, with highest statistical significance for p53 ($p<0.05$). These findings suggest that induction of miR-21, mediated by oxidative stress, might promote tumorigenicity in shFHC-K562 cells through down-regulation of tumor suppressors genes.

O24.3
RNA interference determines nucleosome occupancy at human Transcription Start Sites by interacting with SWI/SNF complex

C. Carissimi[1], I. Laudadio[1], E. Cipolletta[1], S. Gioiosa[1], M. Mihailovich[2], T. Bonaldi[2], G. Macino[1], V. Fulci[1]

[1]Dipartimento di Biotecnologie Cellulari ed Ematologia, "Sapienza" Università di Roma, Rome, Italy, [2]Department of Experimental Oncology, European Institute of Oncology (IEO), Milan, Italy

Argonaute (AGO) proteins have a well-established role in post-transcriptional regulation of gene expression as key component of the RNA silencing pathways. Recent evidence involves AGO proteins in mammalian nuclear processes such as transcription and splicing, though the mechanistic aspects of AGO nuclear functions remain largely elusive. Here, by SILAC based interaction proteomics, we identify the chromatin-remodelling complex SWI/SNF as a novel AGO2 interactor in human cells. Moreover, we show that nuclear AGO2 is loaded with a novel class of DICER-dependent short RNAs, that we called swiRNAs, which map nearby the Transcription Start Sites (TSSs) bound by SWI/SNF. The knock-down of AGO2 decreases nucleosome occupancy at the first nucleosome located downstream of TSSs in a swiRNA-dependent manner. Our findings indicate that in human cells AGO2 binds SWI/SNF and a novel class of sRNAs to establish nucleosome occupancy on target TSSs.

O24.4
LincRNAs landscape in human lymphocytes highlights regulation of T cell differentiation by linc-MAF-4

I. Panzeri, V. Ranzani, G. Rossetti, A. Arrigoni, R. Bonnal, S. Curti, P. Gruarin, E. Provasi, E. Sugliano, B. Bodega, S. Abrignani, M. Pagani

Fondazione Istituto Nazionale Genetica Molecolare, INGM, Milano, Italy

Long intergenic non-coding RNAs (lincRNAs) are a novel class of regulatory RNAs with a high cellular and temporal specificity of expression in defined developmental stages. Recent findings demonstrated the involvement of lincRNAs in cell fate determination and maintenance in diverse biological contexts. Nevertheless only few studies have been published regarding their function in the human immune system. In our work we investigated lincRNAs in thirteen T and B lymphocyte subsets by RNA-seq analysis and de-novo transcriptome reconstruction defining a set of lincRNAs that are specifically expressed in these subsets. In particular, we focused on the $CD4^+$ Th1 lincRNAs signature and investigated the functional role of linc-MAF-4, located 140 Kb upstream to the Th2-associated transcription factor MAF. The expression of linc-MAF-4 is anti-correlated with the expression of MAF and its down-regulation skews T cell differentiation toward a Th2-like fate. We identified a long-distance intra-chromosome interaction between linc-MAF-4 and MAF genomic regions and demonstrated the enrichment of linc-MAF-4 transcript in the chromatin fraction. Furthermore we showed that linc-MAF-4 associates with LSD1 and EZH2, thus likely acting as a recruiter of chromatin modifiers for MAF promoter resetting in Th1 cells. Our results highlight the functional importance of long non-coding RNAs as drivers of human T lymphocytes differentiation processes.

O24.5
O-GlcNAcylation induces hyaluronan synthase 2 transcription modulating promoter chromatin structure via long non coding RNA

D. Vigetti, M. Viola, E. Karousou, Marialuisa D'Amato, G. De Luca, A. Passi
Dept.of Surgery and Morphology Sciences, Univ. of Insubria, Varese, ITALY

The hyaluronan (HA) synthesis is regulated by several factors as covalent modification of the synthetic enzyme (HASes). Recently we demonstrated that the main HA synthase (HAS2), could be controlled by O-GlcNAcylation in human aortic smooth muscle cells (AoSMCs) model. Many cytoplasmic and nuclear proteins, including histones and transcription factors, can be O-glycosylated by O-GlcNAc, therefore we studied whether the expression of the HAS2 could be controlled by O-GlcNAcylation in AoSMCs. We found that the natural antisense transcript (NAT) of HAS2 (HAS2-AS1) was absolutely necessary to induce the transcription of the HAS2 gene. We found that O-GlcNAcylation modulated HAS2-AS1 promoter activation, but not the HAS2 promoter, whereas HAS2-AS1 NAT, working in cis, regulated HAS2 transcription by altering the chromatin structure around the HAS2 proximal promoter via O-GlcNAcylation and acetylation. These results indicate that HAS2 transcription can be finely regulated not only by recruiting transcription factors to the promoter but also by modulating chromatin accessibility by epigenetic modifications.

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