

8th SIBBM Seminar
Frontiers in Molecular Biology

Epigenetics
in
Development and Disease

Programme & Abstracts

Palermo, 24-26 May 2012

Sponsors



Thursday, 24 May

- 14:00-14:15 **Welcome Address**
Giovanni Spinelli (Director of STEMBIO, Palermo)
Valerio Orlando (SIBBM President)
Pier Paolo Di Fiore (ABCD President)

Session I » Environment, Stress & Epigenetics – Chair: Davide Corona (Palermo)

- 14:15-14:55 **Keynote Lecture:** *Sergio Pimpinelli (Rome)* Environmental stress, transposons and evolution
- 14:55-15:20 **EMBO YIP Lecture:** *Fabrizio D'Adda di Fagagna (Milan)* Molecular mechanisms of cellular senescence
- 15:20-15:45 *Stefan Schoeftner (Rome)* ncRNAs tune the function of mammalian telomeres
- 15:45-16:00 *Fabio Ciccarone (Rome)* Poly(ADP-ribosyl)ation acts in DNA demethylation of mouse primordial germ cells through DNA-damage independent roles
- 16:00-16:15 *Angelo Rosa (Trieste)* Structure and dynamics of interphase chromosomes
- 16:15-17:45 **Coffee break and Poster Session I**
- 17:45-18:00 *Dupriez Vincent (Perkin Elmer)* Homogeneous microplate format assays to monitor histone modifications in biochemical and cell-based assays
- 18:00-18:15 *(Merck presentation)*
- 18:15-18:45 **Science & Society Talk:** *Giuseppe Testa (Milan)* Reprogramming genomes and reframing rights
- 18:45-19:15 **Science & Society Talk:** *Enzo De Simone (Naplese)* How (and why) to expose high school students to the emerging concepts at the frontiers of molecular biology: five years experience with the “Eureka” project

Friday, 25 May

Session II » Chromatin Modifications & ncRNA – Chair: Marco Bianchi (Milan)

- 9:00-9:40 **Keynote Lecture:** *Marco Bianchi (Milan)* HMGB1 and the control of nucleosome number
- 9:40-10:05 **Armenise-Harvard Talk:** *Tiziana Bonaldi (Milan)* Mass Spectrometry approach dissects the proteomic landscape of chromatin functional domains
- 10:05-10:20 *Maria Cristina Onorati (Palermo)* An RNA memory mechanism to inherit epigenetic marks
- 10:20-10:35 *Elena Magnani (Busto Arsizio, VA)* CDH1 epigenetic regulation: a role for UHRF1 modulation via promoter associated non-coding RNAs?
- 10:35-11:00 **Coffee break**
- 11:00-11:25 *Davide Gabellini (Milan)* A long non-coding RNA links copy number variation to a Polycomb/Trithorax epigenetic switch in FSHD muscular dystrophy
- 11:25-11:40 *Francesca Munari (Göttingen, Germany)* Molecular basis of hHP1 β /nucleosome interaction in dependence of histone 3 methylation
- 11:40-11:55 *Marco Di Stefano (Trieste)* Gene co-regulation and co-localization in human chromosome 19: a knowledge-based computational approach
- 11:55-12:15 **Progetto Bandiera - Coordinator talk:** *Giuseppe Macino (Rome)* The Italian Epigen Project
- 12:30-14:00 **Lunch break**

Session III » Epigenetic Signaling in Development, Differentiation & Reprogramming – Chair: Giovanni Spinelli (Palermo)

- 14:00-14:40 **EMBO ABCD Lecture:** *Maria Pia Cosma (Barcelona, Spain)* Wnt signalling and the reprogramming of cell fate to pluripotency
- 14:40-15:05 *Michelangelo Cordenonsi (Padua)* The Hippo transducer TAZ confers cancer stem cell traits on breast cancer cells downstream of epithelial-to-mesenchymal transition and the deregulation of the cell polarity determinant Scribble
- 15:05-15:30 *Valerio Orlando (Rome)* Epigenetic control of Repetitive Elements mobilization contributes to cell differentiation and disease
- 15:30-15:45 *Cecilia Battistelli (Rome)* MyoD regulates p57kip2 expression by interacting with a distant cis-element and modifying a higher-order chromatin structure

15:45-16:00 *Paola Tognini (Pisa)* Experience-dependent expression of miR132 regulates ocular dominance plasticity

16:00-16:20 **Editor Talk:** *David del Alamo (The EMBO Journal, Heidelberg, Germany)* Behind the scenes of scientific publication

16:20-17:45 Coffee break and Poster Session II

Session IV » Functional Epigenomics – *Chair: Valerio Orlando (Rome)*

17:45-18:25 **EMBO Lecture:** *Amos Tanay (Rehovot, Israel)* Hi-C and the hierarchical domain chromosomal topology

18:25-18:40 *Raffaele Giancarlo (Palermo)* The chromatin organization of an eukaryotic genome: sequence specific + statistical=combinatorial

18:40-20:00 General SIBBM Society & Board Meeting (SIBBM members only)

20:30 Social dinner (tickets available at SIBBM registration desk)

Saturday, 26 May

Session V » Epigenetics, Disease & Regenerative Medicine

Chair: Irene Bozzoni (Rome)

- 9:00-9:40 **Keynote Lecture:** *Irene Bozzoni (Rome)* The increase in complexity of the RNA landscape: new functions of non coding RNAs
- 9:40-9:55 *Anna Garbelli (Pavia)* A new paradigm for HIV-1 chemotherapy: targeting the host cell viral cofactor DDX3
- 9:55-10:10 *Federica Lo Sardo (Rome)* PcG-mediated higher order chromatin structures modulate replication programs at the *Drosophila* BX-C
- 10:10-10:25 *Anna Comel (Trieste)* Dissecting the tumor suppression activity of the bromodomain containing protein BRD7
- 10:25-10:40 *Italia Anna Asteriti (Rome)* A high-throughput imaging approach to study Aurora-A inhibition in human cell
- 10:40-11:00 Coffee break
- 11:00-11:25 *Gabriella Minchiotti (Naples)* Molecular control of satellite cell lineage progression and muscle regeneration through a novel Cripto -dependent mechanism
- 11:25-11:50 *Vania Broccoli (Milan)* Reprogramming cellular identity for *in vitro* modeling and replacement therapy of Parkinson's disease
- 12:00-12:30 Chiara D'Onofrio "Giovani" Award (prize to be awarded to the best Selected Talk) - Final Remarks & Departure

Oral Presentations

in chronological order of presentation
(presenting authors are shown underlined)

Environmental stress, transposons and evolution

S. Pimpinelli

Dept Biology and e Biotechnology "Charles Darwin", Sapienza Univ., Rome, Italy

After Darwin's book on the origin of species by natural selection, the theory espoused by his predecessor Lamarck was never completely abandoned. To explain, some of the apparent Lamarckian-like phenomena in a Darwinian sense, Waddington elaborated the "canalization and assimilation" concepts (Waddington, *Nature*, 1959). He hypothesised the existence of a cryptic genetic variation that is maintained hidden due to the robustness of the developmental process that he indicated as "canalization". If an environmental stress is strong enough to overcome this robustness, cryptic genetic variants can be expressed and become heritable by an "assimilation" process.

In past years, it has been observed that in flies and plants, when the activity of Hsp90 is reduced, a wide spectrum of phenotypic variants is induced (Rutherford and Lindquist, *Nature*, 1998; Queitsch et al., *Nature*, 2002). The interpretation was that Hsp90 is a "capacitor" of morphological evolution and buffers a cryptic genetic variation that accumulates in neutral conditions.

However, another study by Specchia et al. (*Nature*, 2010) has suggested that Hsp90, rather than functioning as a capacitor, instead acts as a "mutator" through impairment of RNAi silencing. It has been demonstrated that Hsp90 is involved in repression of transcription and mobilization of transposable elements. The reduction of Hsp90 causes stress response-like activation and transposition of mobile elements causing phenotypic variants.

This observation, suggests a mechanism for rapid evolutionary changes: the environmental changes play a direct active role on evolution of genomes by the induction of genetic variability, by means of transposons, and thus allowing for the possibility of selection of more adapted genotypes along with their more adaptive stress-response, i.e. transposons may also make evolvability evolvable.

ncRNAs tune the function of mammalian telomeres

S. Schoeftner

Istituto Nazionale Tumori, Regina Elena (IFO), Rome, Italy

Telomeres are nucleoprotein structures that protect chromosome ends from DNA repair mechanisms. Telomere function is essential for genomic stability and controlled by a delicate equilibrium of the protein complex shelterin, telomerase and the recently discovered non-coding RNA TERRA. Critical telomere shortening in the absence of telomerase triggers senescence and apoptosis. In line with this, re-activation of telomerase activity or unleashing telomere-recombination is a central step in tumorigenesis. Consequently, identifying mechanisms that allow interfering with telomere maintenance is a promising approach to limit cancer cell proliferation.

Non-coding RNAs (ncRNAs) such as miRNAs have been demonstrated to be critically involved into tumorsuppression and oncogenesis. However non-coding RNAs that control telomere function and homeostasis by regulating the expression of shelterin components are unknown. By identifying and characterizing “telo-miRNAs” we wish to uncover new mechanisms of telomere regulation with high relevance to human cancer and organismal ageing.

Poly(ADP-ribosyl)ation acts in DNA demethylation of mouse primordial germ cells through DNA-damage independent roles

F. Ciccarone^{1,2}, F.G. Klinger³, A. Catizone⁴, R. Calabrese^{1,2}, M. Zampieri^{1,2}, M.G. Bacalini^{1,2}, M. De Felici³, P. Caiafa^{1,2}

¹Dept of Cellular Biotechnologies and Hematology, Sapienza Univ. of Rome, Italy

²Pasteur Institute-Fondazione Cenci Bolognetti, Rome, Italy

³Dept of Public Health and Cell Biology, Univ. of Rome Tor Vergata, Italy

⁴Dept of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza Univ. of Rome, Italy

Poly(ADP-ribosyl)ation regulates chromatin structure and transcription driving epigenetic events. In particular, Parp1 is able to directly influence DNA methylation patterns controlling transcription and activity of Dnmt1. Here, we show that ADP-ribose polymers levels and Parp1 expression are noticeably high in mouse primordial germ cells (PGCs) when the bulk of DNA demethylation occurs during germline epigenetic reprogramming in the embryo. Notably, Parp1 activity is stimulated in PGCs also before its participation in the DNA damage response associated with active DNA demethylation. We demonstrate that PARP inhibition impairs both genome-wide and locus-specific DNA methylation erasure in PGCs. Besides acting as DNA break sensor during active DNA demethylation, Parp1 may control the activity of Dnmt1, which is still expressed in PGCs undergoing reprogramming. Moreover, we evidence that impairment of PARP activity causes a significant reduction of the expression of genes coding for Tet hydroxylases involved in active DNA demethylation. Taken together these results demonstrate new and adjuvant roles of poly(ADP-ribosyl)ation during germline DNA demethylation and suggest its possible more general involvement in genome reprogramming.

Structure and dynamics of interphase chromosomes

A. Rosa¹, R. Everaers²

¹Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

²Ecole Normale Supérieure de Lyon, Lyon, France

During interphase chromosomes decondense, but fluorescent in situ hybridization experiments reveal the existence of distinct territories occupied by individual chromosomes inside the nuclei of most eukaryotic cells. We use computer simulations to show that the existence and stability of territories is a kinetic effect that can be explained without invoking an underlying nuclear scaffold or protein-mediated interactions between DNA sequences. In particular, we show that the experimentally observed territory shapes and spatial distances between marked chromosome sites for human, fruit-fly, and budding yeast chromosomes can be reproduced by a parameter-free minimal model of decondensing chromosomes.

Our results suggest that the observed interphase structure and dynamics are due to generic polymer effects: confined Brownian motion conserving the local topological state of long chain molecules and segregation of mutually unentangled chains due to topological constraints.

References:

- 1) A. Rosa, R. Everaers, *Plos Comput. Biol.* 4, e1000153 (2008)
- 2) A. Rosa et al., *Biophys. J.* 98, 2410 (2010)

Reprogramming genomes and reframing rights

G. Testa

European Institute of Oncology, Milan, Italy

In this work I trace the mutual shaping between the emergence of cell technologies that reprogram our bodily lineages and the legal and political settlements that reframe rights and institutions around their circulation. The ability to extract cells from organisms and grow them in vitro is a foundational resource in the life sciences, aiming at modeling, reproducing or managing living processes under increasingly defined conditions. More recently, the emerging field of systems biology has been attempting a new modeling leap, with the ambition to derive in silico models that are mostly generated from cells grown in vitro and whose interpretation is related back to living functions. This triangulation between in vivo, in vitro and in silico is now receiving a paradigm-shift with the advent of cell fate reprogramming induced by transcription factors, translating into the ability to generate, from any individual, a virtually limitless supply of cells growing in vitro into possibly any desired lineage. Modeling ambitions are therefore contemplating new heights, as testified by ‘disease in a dish’ approaches being applied to model also complex behavioral traits. Yet, the increasing ability to manipulate cell fate has opened a space of experimentation that is not only epistemic but also explicitly social. For, as cells are sourced from bodies to model various aspects of the human condition in their circulation through academic labs, companies, biobanks and hospitals, they become key resources for the operation of current life sciences in their molecular gaze on humanness. And as such they become invested with the political, moral and economic rationalities that inspire their generation, thus becoming structuring elements of a normative order on the human condition and its underlying dichotomies in knowledge-intensive societies: normal versus pathological; natural versus artificial; private versus public; individual versus collective; expert versus lay.

HMGB1 and the control of nucleosome number

B. Celona¹, A. Weiner², F. Di Felice³, F. Mancuso⁴, E. Cesarini³, R.L. Rossi⁵, L.Gregory⁶, D. Baban⁶, G. Rossetti⁵, M. Pagani⁵, Giovanni Pietrogrande¹, T. Bonaldi⁴, I. Ragoussis⁶, N. Friedman², G. Camilloni³, A. Agresti¹, M.E. Bianchi¹

¹San Raffaele Univ. and Research Institute, Milan

²Hebrew Univ., Jerusalem

³Univ. di Roma La Sapienza, Rome

⁴IFOM-IEO Campus, Milan

⁵Istituto Nazionale Genetica Molecolare, Milan

⁶Univ. of Oxford, Oxford

It is currently assumed that the number of nucleosomes is fixed, and proportional to the quantity of DNA to be packaged. The location of some nucleosomes may depend on the sequence being packaged (or its propensity to being bent). Strongly positioned nucleosomes may determine the "statistical positioning" of the remaining nucleosomes, like some well-parked cars guide the parking of other cars in a parking lot. Nucleosome position determines the accessibility of DNA, and controls gene expression.

We show that High Mobility Group Box 1 protein (HMGB1) facilitates nucleosome assembly. Mammalian cells lacking HMGB1 contain a reduced amount of core, linker and variant histones, and a correspondingly reduced number of nucleosomes. Yeast *nhp6* mutants lacking Nhp6a and -b proteins, which are related to HMGB1, also have a reduced amount of histones and fewer nucleosomes. Nucleosome limitation in both mammalian and yeast cells increases the sensitivity of DNA to damage, increases transcription globally, and affects the relative expression of about 10% of genes. In yeast *nhp6* cells the loss of more than one nucleosome in four does not affect the location of nucleosomes and their spacing, but nucleosomal occupancy. The decrease in nucleosomal occupancy is non-uniform; nucleosomes on sites with low propensity to occupation are disproportionately lost in nucleosome-depleted cells.

We disprove the notion that the number of nucleosomes is fixed and set by default. At least in yeast, variation in nucleosome number affects nucleosomal occupancy (but not location), both genomewide and gene-specifically, and constitutes a novel layer of epigenetic regulation.

A combined ChIP - Mass Spectrometry approach dissects the proteomic landscape of chromatin functional domains

M. Soldi and T. Bonaldi*

European Institute of Oncology (IEO), Dept of Experimental Oncology, c/o IFOM-IEO Campus, Milan, Italy

Chromatin is a highly dynamic well organized nucleoprotein complex of DNA and proteins that controls various DNA-dependent processes. Chromatin dynamicity is regulated by various chromatin associated proteins, post-translational modifications of histones and DNA methylation; however, until now the characterization of the proteomic component of chromatin at specific regions has been held back by the difficulty in enriching such domains at purity and amount sufficient for the in depth analysis of proteins and hPTMs associated in a combinatorial fashion. We describe here the optimization of a method for a preparative-ChIP to isolate specific functional chromatin regions and the subsequent mass spectrometric analysis to identify their interactome and hPTMs patterns. Our *PROMIC* (Proteomic Mapping of Immunopurified Chromatin) approach leads to the characterization of transcriptionally silent and active chromatin regions, marked by H3K9me3 and H3K4me3, respectively and expands the arsenal of analytical strategies aiming at understanding how hPTMs combinations and their specific interactors mediate the locus-specific structural-function of chromatin.

An RNA memory mechanism to inherit epigenetic marks

M.C. Onorati¹, W. Arancio², D.F.V. Corona¹

¹STEMBIO Dept Dulbecco Telethon Institute c/o Univ. of Palermo

²Endocrinologia e malattie metaboliche Azienda Universitaria Policlinico PAolo Giaccone, Palermo

A central question in epigenetics is to understand how, terminally differentiated daughter cells can inherit complex patterns of chromatin modifications from their mother cell. Even if several mechanisms have been hypothesized to explain the establishment and maintenance of cell identity, it is still unclear how during mitosis covalent and ATP-dependent chromatin modifications are transmitted after DNA replication. Indeed, a simple way for daughter cells to restore the transcriptional profile of mother cells is to directly 'sense' the transcriptome of their mother cells.

In order to unveil the molecular nature of somatic cell epigenetic memory, we used classic Position Effect Variegation assays to check if non functional alleles of the white gene could modify the eye color variegation caused by a heterochromatin inversion of the white gene called white-mottled 4 (wm4h). Our data show that several white alleles suppress the variegation of the wm4h line. Unexpectedly, the presence of white alleles causes an increase in the white gene transcript as well as an opening in the chromatin structure at the wm4h locus. Remarkably, this effect is inheritable, a phenomenon highly reminiscent of RNA mediated paramutation. The changes in the levels of expression of the wm4h gene, induced in trans by several white alleles, indicate that the presence of a non functional gene that does not produce a coding transcript but potentially only ncRNA, could influence in trans the expression of a functional copy of the same gene silenced by heterochromatin. Our data indirectly indicates that cells can 'sense' the presence of non coding RNA's inherited from their mother cells and can use them to epigenetically reset their transcriptional program after DNA replication.

CDH1 epigenetic regulation: a role for UHRF1 modulation via promoter associated non-coding RNAs?

C. Pistore¹, E. Magnani¹, A. Garzia¹, F. Babbio¹, G. Carbone², C. Catapano², I.M. Bonapace¹

¹Dept of Theoretical and Applied Sciences, Univ. of Insubria, Busto Arsizio (VA), Italy

²Institute of Oncology Research (IOR), Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Switzerland

Epigenetic silencing of tumour suppressor genes is an important step during tumorigenesis. This process involves several mechanisms including DNA methylation and histone modifications. UHRF1 is a multi-domain protein able to recognize both DNA and histone silencing marks, coupling the preservation of histone-modification through the cell cycle with maintenance of DNA methylation. Recently it has been demonstrated that UHRF1 is over-expressed in several tumours and that its over-expression correlates to tumour progression. In the last years several evidences showed that also non-coding RNAs (ncRNAs) play a pivotal role in epigenetic regulation. In particular, it has been demonstrated that ncRNAs mediate the epigenetic silencing of many genes, in a process termed transcription gene silencing.

In this work we demonstrated that, in prostate cancer cell lines, UHRF1 mediates epigenetic silencing of many tumour suppressor genes such as, E-cadherin gene CDH1, through the binding with its promoter and the recruitment of the histone-methyltransferase Suv39H1. Our data demonstrated that the aggressive androgen-independent cells (PC3) express high levels of UHRF1, while the low tumorigenic androgen-dependent cells (LNCaP) show low levels of UHRF1, according to the low and high level of CDH1, respectively. Then, we demonstrated that PC3 and LNCaP express two promoter associated ncRNAs, sense and antisense directed, transcribed from CDH1 promoter. In particular, LNCaP showed both sense and anti-sense ncRNAs, whereas PC3 cells expressed only sense ncRNA. In PC3 the knockdown, through targeted siRNAs, of UHRF1 impaired Suv39H1 binding to the CDH1 promoter and induced CDH1 and antisense-directed promoter associated ncRNA re-expression. Supported by these data we can hypothesize that in prostate cancer cells not only UHRF1 but also promoter-associated ncRNAs are involved in CDH1 transcriptional silencing and they could be part of the same regulatory network.

A long non-coding RNA links copy number variation to a Polycomb/Trithorax epigenetic switch in FSHD muscular dystrophy

D. Gabellini

Dulbecco Telethon Institute at San Raffaele Scientific Institute, Division of Regenerative medicine, Stem cells, and Gene therapy, Milan, Italy

Repetitive sequences account for more than 50% of the human genome. While once thought of primarily as “junk”, recent studies indicate that repeated elements play central roles in regulating gene expression at multiple levels. Interestingly, in mammals the bulk of several epigenetic marks is located in genomic repeats and it has been suggested that they could provide binding platforms for chromatin regulators. Hence, elucidating the role of these elements in setting up functional chromatin states in complex genomes is of paramount importance.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease associated to reduction in the copy number of the D4Z4 repeat mapping to 4q35. By an unknown mechanism, D4Z4 deletion causes an epigenetic switch leading to de-repression of 4q35 genes. We have that the Polycomb group of epigenetic repressors targets D4Z4 in healthy subjects and that D4Z4 deletion is associated with reduced Polycomb silencing in FSHD patients. We have identified *DBE-T*, a chromatin-associated non-coding RNA produced selectively in FSHD patients that coordinates de-repression of 4q35 genes. *DBE-T* recruits the Trithorax group protein Ash1L to the FSHD locus, driving histone H3 lysine 36 dimethylation, chromatin remodeling and 4q35 gene transcription.

Our study provides insights into the biological function of repetitive sequences in regulating gene expression and shows how mutations of such elements can influence the progression of a human genetic disease.

Molecular basis of hHP1 β /nucleosome interaction in dependence of histone 3 methylation

F. Munari¹, S. Soeroes^{2,6}, H. M. Zenn³, A. Schomburg^{2,6}, N. Kost², S. Schröder^{2,4}, R. Klingberg⁵, N. Rezaei-Ghaleh¹, A. Stützer², K. A. Gelato², P. J. Walla⁴, S. Becker¹, D. Schwarzer⁵, B. Zimmermann³, W. Fischle², M. Zweckstetter¹

¹Dept for NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

²Laboratory of Chromatin Biochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

³Biaffin GmbH & Co KG, Kassel, Germany

⁴Biomolecular Spectroscopy and Single-Molecule Detection, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

⁵Protein Chemistry, Leibniz-Institut für molekulare Pharmakologie, Berlin, Germany

⁶Current Address: Oxford Nanopore Technologies LTD, Oxford, United Kingdom (SS), Proteros biostructures GmbH, Martinsried, Germany (AS)

A large variety of post-translational histone modifications regulate chromatin structure and function. Methylation of lysine 9 within the H3 N-terminus governs the packaging of DNA into heterochromatin, a highly condensed and gene-silent form of chromatin. The recognition of trimethylated Lys9 in histone3 (H3K9me3) by heterochromatin proteins 1 (HP1) constitutes a key step in the establishment and maintenance of heterochromatin¹.

Previous works on component systems using isolated histone peptides and/or individual domains of the protein^{2,3,4}, as well as using HP1 proteins of different species or isoforms^{5,6,7}, have determined various domains and interaction interfaces of HP1 binding to chromatin. The large variability of findings precludes from deducing a coherent picture of HP1/nucleosome binding. In particular structural and biophysical insights have been missing in this picture so far. By using a combination of NMR, biophysical and biochemical experiments on a fully defined recombinant system we have investigated the molecular basis of hHP1 β binding to methylated (H3K9me3) and unmodified nucleosomes. Our results provide the first detailed analysis of the dynamic, structural and thermodynamic parameters of hHP1 β binding to nucleosome in dependence of K9 methylation on histone 3.

1. Grewal SI, Elgin SC. *Nature* 447: 399-406; 2007
2. Nielsen PR, et al. *Nature* 416: 103-107; 2002
3. Nielsen AL, et al. *Mol Cell* 7: 729-739; 2001
4. Lavigne M, et al. *PLoS Genet* 5: e1000769; 2009
5. Meehan RR, et al. *Embo J* 22: 3164-3174; 2003
6. Zhao T, et al. *J Biol Chem* 275: 28332-28338; 2000
7. Canzio D, et al. *Mol Cell* 41: 67-81; 2011

Gene co-regulation and co-localization in human chromosome 19: a knowledge-based computational approach

M. Di Stefano¹, A. Rosa¹, V. Belcastro², D. di Bernardo³, C. Micheletti¹

¹International School for Advanced Studies (SISSA), Trieste, Italy

²Philip Morris International R&D, Philip Morris Products S.A., Neuchatel, Switzerland

³Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy

Clarifying the link between transcriptional regulation and 3D chromatin organization is one of the major open issues in molecular biology. Here, we tackle “in-silico” a specific aspect of this complex relationship, namely that the spatial proximity of chromatin fibers should enhance co-regulation of the corresponding hosted genes (“gene kissing” hypothesis, G. Cavalli, *Curr. Opin. Genet. Dev.* 2007).

Specifically, we have extended the computational polymer model for interphase chromosomes recently proposed by Rosa and Everaers (*Plos Comput. Biol.*, 2008), by including putative propensities for gene-gene contacts based on co-expression data. The gene-gene contacts were promoted through a steered molecular dynamics protocol on a model chromatin fiber. The resulting “in silico” structural organization of the fiber shows folding motifs which should be detected by current experimental techniques. Our model helps in formulating novel hypotheses on the structure-function relationship of the chromatin.

Wnt signalling and the reprogramming of cell fate to pluripotency

M.P. Cosma

Centre for Genomic Regulation (CRG), Barcelona, Spain

Spontaneous cell fusion between two cells of different lineages can originate new hybrid cells that have different features from the original parent cells. If one of the fusing parent cells is highly plastic, such as a stem cell, and the other is a somatic cell, their fusion can be followed by reprogramming events that generate new hybrid pluripotent cells. However, whether cell-fusion-mediated reprogramming can occur *in vivo* in higher vertebrates, and what are the molecular mechanisms and genes that drive the reprogramming, remain to be defined. We have shown that activation of the Wnt/ β -catenin signalling pathway enhances reprogramming of somatic cells after their fusion with embryonic stem cells. We are currently dissecting out the gene networks and studying the mechanisms of *in-vivo* somatic-cell reprogramming, to determine whether reprogrammed hybrids have the potential to differentiate and regenerate tissues.

The Hippo transducer TAZ confers cancer stem cell traits on breast cancer cells downstream of epithelial-to-mesenchymal transition and the deregulation of the cell polarity determinant Scribble

M. Cordenosi, F. Zanconato, L. Azzolin, S. Piccolo

Dept of Biomedical Sciences, Univ. of Padua, Padova, Italy

Cancer Stem Cells (CSCs) are proposed to drive tumor initiation, heterogeneity and progression. Yet, our understanding of the cellular and molecular mechanisms that underlie CSC properties is limited. We present evidences indicating that TAZ, a transducer of the Hippo pathway, is a determinant of key biological traits of breast CSCs. TAZ is required to sustain self-renewal and tumor initiation capacities in cellular models of breast cancer progression. TAZ protein levels are stabilized in prospective CSCs, and gain-of-TAZ in non-CSCs induces them to adopt CSCs-like behaviors. We found that gene-signatures denoting TAZ activity are associated with molecular imprints of "stemness" in breast cancer patients' datasets. In agreement with the view that an increase in CSCs drives tumor progression in breast cancer, the proportion of TAZ positive cells is much more abundant in poorly-differentiated tumors than in well differentiated ones. Consistently, raising TAZ levels promotes the transition of experimentally induced tumors toward a less-differentiated status. At the molecular level, TAZ promotes self-renewal of CSC downstream of Epithelial-to-Mesenchymal Transition (EMT). This is caused by deregulation of the cell polarity determinant Scribble, which forms an endogenous complex with TAZ in nontransformed and tumoral mammary epithelial cells. Remarkably, loss-of-Scribble - or induction of EMT - disrupts the association of TAZ with the core Hippo kinases MST and LATS, allowing TAZ to escape phosphorylation by LATS and association to beta-TrCP ubiquitin ligase complex. This study thus links the CSCs concept to the Hippo pathway in breast cancer, and reveals a mechanistic basis of the control of Hippo kinases by cell polarity.

Epigenetic regulation of L1 repetitive elements plays a role in normal myogenesis and is altered in Duchenne muscular dystrophy

B. Bodega¹, F. Della Valle¹, V. Saccone^{2,3}, S. Consalvi^{2,3}, J. Martone⁴, V. Cazzella⁴, C. Mozzetta², M. Mora⁵, P. Carninci⁶, I. Bozzoni⁴, P.L. Puri^{2,3}, V. Orlando¹

¹Dulbecco Telethon Institute (DTI) at IRCCS Fondazione Santa Lucia, Epigenetics and Genome reprogramming, Rome, Italy

²Dulbecco Telethon Institute (DTI), IRCCS Fondazione Santa Lucia, Rome, Italy

³Sanford-Burnham Institute for Medical Research, La Jolla, USA

⁴Dept of Biology and Biotechnology "Charles Darwin", Sapienza Univ. of Rome, Italy

⁵Division of Neuromuscular Diseases and Neuroimmunology, Istituto Nazionale Neurologico 'C. Besta', Milano, Italy

⁶RIKEN Yokohama Institute, Omics Science Center, Yokohama, Kanagawa, Japan

The regulation and impact of Repetitive Elements in human genome function and disease is largely unknown. Duchenne muscular Dystrophy is one of the most debilitating myopathies in humans and no effective therapies are available for this disease. Emerging findings suggest that lack of dystrophin, part of the dystrophin-associated protein complex (DAPC) at the sarcolemma, affects not only the structure of muscle fibers, but has a consistent impact on global genome expression regulation (i.e. protein coding genes and miRNAs), acting on the nNOS-HDAC2 pathway 2,3,4. Here we report that the same pathway modulates somatic LINE-1 (L1) expression and copy number variation (CNV) during skeletal myogenesis, concomitantly with L1 promoter hyper-acetylation and MyoD1 binding. Small molecule inhibition of CNV reduced differentiation ability of normal muscle cells suggesting a role for L1 mobilization in normal myogenesis. We detected HDAC2 aberrant recruitment on L1 promoter, together with lower L1 expression and no CNV in DMD muscle cells. Notably, amelioration of the dystrophic features using HDAC inhibitors (TSA) or exon-skipping dystrophin rescue restore L1 expression and CNV either in the mdx mouse model of DMD and in human DMD muscle cells. These results demonstrate that epigenetic regulation of L1 sequences occurs in muscle cell progenitors and may be a requirement for myogenesis in mammals. Moreover, we unravel a direct link between absence of Dystrophin and defective HDAC2 regulated L1 retrotransposition in DMD patients and identify major human non coding sequences as potential new players in muscular dystrophy manifestation. These data suggest the relevance of studying non coding genome activity to fully understand human diseases.

MyoD regulates p57kip2 expression by interacting with a distant cis-element and modifying a higher-order chromatin structure

C. Battistelli, A. Busanello, M. Carbone, C. Mostocotto, R. Maione

Istituto Pasteur-Fondazione Cenci Bolognetti, Dept Cellular Biotechnologies and Haematology, Sapienza Univ. of Rome, Italy

The bHLH transcription factor MyoD, the prototypical master regulator of differentiation, directs a complex program of gene expression during skeletal myogenesis. The up-regulation of the cdk inhibitor p57kip2 plays a critical role in coordinating differentiation and growth arrest during muscle development as well as in other tissues. p57kip2 displays a highly specific expression pattern and is subject to a complex epigenetic control driving the imprinting of the paternal allele. However, the regulatory mechanisms governing its expression during development are still poorly understood. We have identified an unexpected mechanism by which MyoD regulates p57kip2 transcription in differentiating muscle cells. We show that the induction of p57kip2 requires MyoD binding to a long-distance element located within the imprinting control region KvDMR1 and the consequent release of a chromatin loop involving p57kip2 promoter. We also show that differentiation-dependent regulation of p57kip2, while involving a region implicated in the imprinting process, is distinct and hierarchically subordinated to the imprinting control. These findings highlight a novel mechanism, involving the modification of higher order chromatin structures, by which MyoD regulates gene expression. Our results also suggest that chromatin folding mediated by KvDMR1 could account for the highly restricted expression of p57kip2 during development and, possibly, for its aberrant silencing in some pathologies.

Experience-dependent expression of miR132 regulates ocular dominance plasticity

P. Tognini^{1,2}, E. Putignano^{1,2}, A. Coatti^{1,2}, D. Silingardi¹, R. Mazziotti¹, D. Napoli¹, T. Pizzorusso^{1,3}

¹Institute of Neuroscience CNR, Pisa, Italy

²Scuola Normale Superiore, Pisa, Italy

³Dipartimento di Psicologia, Univ. di Firenze, Firenze, Italy

Brain development is characterized by temporal windows of enhanced experience-dependent plasticity called critical periods. Ocular dominance (OD) plasticity induced by monocular deprivation during the critical period is a classical model of developmental plasticity. Visual activation of signalling pathways converging on CREB and on histone post-translational modifications regulate visual cortical plasticity. Little is known about the plasticity genes controlled by these mechanisms. We investigated the role of miR132, a CREB induced microRNA controlling spine formation, in visual cortical plasticity. We found that visual experience dynamically regulated primary and mature miR132 expression and induced methylation and phosphoacetylation of histones on a CRE locus important for miR132 transcription in the visual cortex. Monocular deprivation reduced primary and mature miR132 expression in the cortex contralateral to the deprived eye. Counteracting miR132 reduction with infusion of chemically modified miR132 mimic oligonucleotides completely blocked OD plasticity. Furthermore, three days of monocular deprivation in miR212/132 KO mice didn't induce OD shift during critical period. Thus, developmental cortical plasticity requires experience-dependent regulation of miR132 expression.

The chromatin organization of an eukaryotic genome: sequence specific + statistical=combinatorial

D. Corona¹, V. Di Benedetto², R. Giancarlo², F. Utro³

¹Dulbecco Telethon Institute c/o Università di Palermo, Dipartimento STEMBIO – Sezione Biologia Cellulare, Palermo, Italy

²Univ. di Palermo, Dipartimento di Matematica ed Informatica, Palermo, Italy

²Univ. di Palermo, Dipartimento di Matematica ed Informatica, Palermo, Italy

³Computational Genomics Group, IBM T.J. Watson Research Center, Yorktown Heights, USA

Nucleosome organization in eukaryotic genomes has a deep impact on gene function. Although progress has been recently made in the identification of various concurring factors influencing nucleosome positioning, it is still unclear whether nucleosome positions are sequence dictated or determined by a random process. It has been postulated for a long time that, in the proximity of TSS, a ‘barrier’ determines the position of the +1 nucleosome and then geometric constraints alter the random positioning process determining nucleosomal phasing. Such a pattern fades out as one moves away from the barrier to become again a random positioning process. Although this statistical model is widely accepted, the molecular nature of the ‘barrier’ is still unknown. Moreover, we are far from the identification of a set of sequence rules able: to account for the genome-wide nucleosome organization; to explain the nature of the barriers on which the statistical mechanism hinges; to allow for a smooth transition from sequence-dictated to statistical positioning and back. Here we show that sequence complexity, quantified via various methods, can be the rule able to at least partially account for all of the above. In particular, we have conducted our analyses on four high resolution nucleosomal maps of the model eukaryotes *S.cerevisiae*, *C. elegans* and *D.melanogaster*, and found that nucleosome depleted regions can be well distinguished from nucleosome enriched regions by sequence complexity measures. In particular, the depleted regions are less complex than the enriched ones. Moreover, around TSS, complexity measures alone are in striking agreement with in vivo nucleosome occupancy, in particular precisely indicating the positions of the +1 and -1 nucleosomes. Those findings indicate that the intrinsic richness of subsequences within sequences plays a role in nucleosomal formation in genomes, and that sequence complexity constitutes the molecular nature of nucleosome ‘barrier’.

The increase in complexity of the RNA landscape: new functions of non coding RNAs

M. Cesana, D. Cacchiareli, V. Cazzella, M. Morlando, J. Martone, C. Pinnarò, I. Legnini,
I. Bozzoni

Dept of Biology and Biotechnology, Sapienza Univ., Rome

It is becoming largely accepted that the non-coding portion of the genome, and not the coding counterpart, is likely to account for the greater complexity of higher eukaryotes. Many new functions have been assigned to non-coding RNAs both in the nucleus and in the cytoplasm. Among the small non-coding RNAs a relevant role is certainly played by microRNAs. In animals, they are implicated in an increasingly wide variety of biological processes and their expression appears to be tissue-specific and highly regulated according to the cell's developmental lineage and stage. While thousands of mammalian genes are potentially targeted by miRNAs, the functions of miRNAs in the context of gene networks controlling cell differentiation is a relevant issue awaiting a complete definition. However, it is becoming clear that these molecules occupy very high hierarchical positions in the cascade of regulatory events controlling cell specification.

Likewise what happened for the very well known small non-coding RNAs, long non-coding RNAs (lncRNAs) are now attracting much interest.

Even if a large fraction of lncRNAs has not been yet characterized, several general features have been identified indicating that they should play physiological relevant functions and may represent a major regulatory component of the eukaryotic genome (Mattick, 2011, FEBS Letters 585: 1600). The discovery of biologically meaningful lncRNAs provides an additional layer of gene expression regulation at the level of chromatin modification, transcription and post-transcriptional control.

Among the many different functions, one resulted particularly attractive: coding and non-coding RNAs were described to regulate one another through their ability to compete for microRNA binding; these molecules have been termed competing endogenous RNA (ceRNA). ceRNAs can sequester microRNAs, thereby protecting their target mRNAs from repression (Salmena et al., 2011, Cell, 146, 353-358).

Data will be presented on the relevant role of both small and long non-coding RNAs in proper cell differentiation programmes (Cesana et al., 2011, Cell, 147: 358-369).

A new paradigm for HIV-1 chemotherapy: targeting the host cell viral cofactor DDX3

A. Garbelli¹, S. Beermann², G. Di Cicco¹, U. Dietrich², G. Maga¹

¹Institute of Molecular Genetics, National Research Council, Pavia, Italy

²Georg-Speyer-Haus Institute of Biomedical Research, Frankfurt, Germany

DEAD-box proteins are enzymes endowed with nucleic acid-dependent ATPase and unwinding activities. The human DEAD-box protein DDX3 has been shown to play important roles in tumor proliferation and viral infections. For this reason, DDX3 seems a prime target for viral manipulation, however only limited information is available on its biochemical properties. We have started to characterize the biochemical properties of DDX3, as a necessary step for the subsequent development of specific inhibitors of its enzymatic activities.

In particular we investigated the functional role of a unique insertion between motifs I and Ia of DDX3 and provide evidence for its distinct functions in mediating the interaction with nucleic acids and in coupling RNA and DNA binding to ATP hydrolysis. We also show that human DDX3 can act as a DNA helicase with a 5'->3' directionality and provide evidence for an important role of the DDX3 specific module for this activity.

Next, given the role of DDX3 in the HIV-1 life cycle, we aimed to develop small-molecules inhibitors of DDX3 as potential anti-HIV agents. In fact, compounds currently used for the treatment of HIV-1 infections are targeted to viral proteins. However, cellular cofactors represent attractive new targets for HIV-1 chemotherapy, since they should be less prone to accumulate drug resistance mutations.

We have identified the first non-nucleosidic compounds suppressing HIV-1 replication by targeting the ATPase activity of human DDX3. A hit optimization protocol was applied to these compounds which led to design and synthesis of second generation derivatives with better inhibitory activity toward the cellular DDX3 enzyme and HIV-1 replication. These results provide a proof-of-principle for the feasibility of blocking HIV-1 infection by rendering the host cell environment less favorable for the virus.

PcG-mediated higher order chromatin structures modulate replication programs at the *Drosophila* BX-C

F. Lo Sardo^{1*}, C. Lanzuolo^{2*}, F. Comoglio⁴, M. de Bardi³, R. Paro^{4,5}, V. Orlando¹

¹Dulbecco Telethon Institute at IRCCS Santa Lucia Foundation Epigenetics and Genome Reprogramming, Rome, Italy

²CNR Institute of Cellular Biology and Neurobiology, IRCCS Santa Lucia Foundation, Rome, Italy

³Neuroimmunology Unit, Santa Lucia Foundation at the European Brain Research Institute, Rome, Italy

⁴Dept of Biosystems Science and Engineering, Swiss Federal Institute of Technology Zürich, Basel, Switzerland

⁵Faculty of Science, Univ. of Basel, Basel, Switzerland

Polycomb group of proteins (PcG) are conserved transcriptional regulators required for the maintenance of repressed transcriptional states, acting directly on specific chromatin regions via post-translational histone modifications and higher order structures formation. During S-phase, in addition to DNA, PcG mediated epigenetic signatures need to be duplicated to preserve the cell identity, requiring a tight coordination between PcG proteins and replication programs. However, to date the mutual regulation between replication and PcG-functions is still unknown. To address this issue we used *Drosophila* embryonic cell lines, performing genome-wide bioinformatic analyses followed by functional experiments on the well characterized BX-C locus. We found that transcription per se is not the sole determinant of cellular replication timing, whereas higher order structures can dictate the timing of replication. In particular, using available datasets, we show that, at a genome-wide scale, PRC1 and PRC2 complexes differently correlate with replication timing of their targets. On the other hand, at the repressed BX-C, where both complexes are bound, loss of function experiments followed by replication timing analysis revealed a synergistic role for PcG proteins in the maintenance of replication programs through the mediation of BX-C higher order structures, as measured by Chromosome Conformation Capture. In line with this observation, replication timing analysis performed on two *Drosophila* cell lines presenting different BX-C conformations, revealed a cell type specific replication program that mirrors the characteristic BX-C higher order structures. Our work suggests that PcG complexes, by modulating higher order chromatin structure at their target sites, contribute to the definition and the maintenance of genomic structural domains where genes that are co-regulated replicate at the same time.

Dissecting the tumor suppression activity of the bromodomain containing protein BRD7

A. Comel^{1,2}, F. Mantovani^{1,2}, G. Del Sal^{1,2}

¹Dept Life Sciences, Trieste Univ., Trieste, Italy

²Laboratorio Nazionale CIB, Area Science Park, Padriciano, Trieste, Italy

The p53 family proteins, namely p53, p63 and p73, are key inhibitors of tumor development and progression. Among p53's major anticancer responses are oncogene-induced senescence and DNA damage-induced apoptosis; moreover, p53 also promotes DNA repair, inhibits angiogenesis, alters energy metabolism and regulates stemness. All these activities are mainly achieved through p53's transcriptional activity that is finely modulated by several p53-interacting partners in response to both physiological inputs and to oncogenic stress. Our research group has recently demonstrated that the bromodomain-containing protein BRD7, a component of chromatin remodeling complexes, acts as a transcriptional cofactor of wild-type p53 in promoting the expression of genes that regulate growth arrest and DNA repair. BRD7 appears to be essential for protecting normal human fibroblasts from oncogene-induced transformation and we found low BRD7 expression specifically in a subgroup of human breast tumors harboring wild-type, but not mutant, p53. Notably, BRD7 was shown to interact with the tumor suppressor protein BRCA1 and to co-regulate a subset of target genes, among which the DNA repair factor Rad51 and the estrogen receptor α . Moreover, it has recently emerged that BRD7 regulates the expression of the Bmi-1 oncogene, required for self-renewal properties of stem cells. All the above findings point towards a more general role of BRD7 in modulating the transcription of genes involved in cancer development and progression. In particular, gene expression analysis of human fibroblasts upon HRASV12 induction and BRD7 depletion suggests a possible involvement of BRD7 in regulating processes such as cell motility, energy metabolism and secretion of important cell-signaling mediators. Therefore, we took advantage of these observations to investigate the role of BRD7 loss in specific steps of breast cancer progression, with particular relevance to acquisition of metastatic and stem-cell properties.

A high-throughput imaging approach to study Aurora-A inhibition in human cells

I.A. Asteriti, E. Di Cesare, P. Lavia, G. Guarguaglini

CNR Institute of Molecular Biology and Pathology (IBPM), c/o Sapienza Univ. di Roma, Roma, Italy

The faithful segregation of the replicated genomes in mitosis requires an intricate regulatory programme for the cyclic build-up of a functional mitotic spindle. Misassembly or malfunction of the mitotic spindle can yield imbalanced chromosome segregation, aneuploidy and hence cell transformation. Many of the underlying processes are driven by phosphorylation events orchestrated by mitotic kinases, some of which are abnormally expressed in cancer cells and are regarded as attractive targets in cancer therapy. Aurora-A, a mitotic kinase regulating several aspects of spindle assembly, is overexpressed in many tumor types. Using siRNA-based post-transcriptional silencing, we have recently characterised a number of molecular processes regulated by Aurora-A, including the organisation of spindle poles and the balance of forces within the spindle. We have now developed *in vivo* imaging methods to record the fate of living cells after treatment with MLN8237, a highly specific inhibitor of Aurora-A currently under clinical trial. Our results depict unforeseen, potentially dangerous effects of Aurora-A inhibition-based therapeutic protocols which could not have been revealed using conventional cell molecular methods. This work highlights the power of *in vivo* imaging protocols to 1) unravel the biological effects of Aurora-A inhibition, 2) depict the origin of defects at the single cell level which may originate a genetically unstable somatic cell clone and 3) identify conditions that may influence the outcome of Aurora-A targeted therapy.

This work was developed in part as a proof-of-concept study in the EuroBioImaging consortium (EMBL, Heidelberg) and was supported in part by a SIBBM grant award to LAA.

Molecular control of satellite cell lineage progression and muscle regeneration through a novel Cripto -dependent mechanism

G. Minchiotti

Stem Cell Fate Laboratory, Institute of Genetics and Biophysics "A. Buzzati-Traverso", CNR, Naples

Skeletal muscle regeneration mainly depends upon satellite cells, a population of resident muscle stem cells. Despite extensive studies, the molecular mechanisms underlying satellite cell mobilization and skeletal muscle regeneration still remain undefined. We have identified the EGF-CFC protein Cripto as a new, unpredicted player of skeletal muscle regeneration. Cripto is a multifunctional protein acting as a modulator of the TGF β family signalling pathway. Despite the well-described role of Cripto in early embryogenesis and in the multiple signalling networks that orchestrate Embryonic Stem Cell (ESC) differentiation, its function in adult life remains elusive. We provide unprecedented evidence that Cripto is re-expressed in the early phases of skeletal muscle regeneration, in regenerating myofibers. Conditional inactivation of cripto in adult satellite cells compromises skeletal muscle regeneration, whereas gain of function of Cripto accelerates regeneration leading to muscle hypertrophy. Moreover, we show that Cripto modulates myogenic cell determination and promotes proliferation of satellite cells by antagonizing the TGF β ligand Myostatin. Our data provide new insights in the molecular and cellular basis of Cripto activity in skeletal muscle regeneration and satellite cell mobilization, raising novel implications for stem cell biology and regenerative medicine.

Reprogramming cellular identity for in vitro modeling and replacement therapy of Parkinson's disease

M. Caiazzo¹, M.T. Dell'Anno¹, I. Theka¹, S. Curreri², D. Leo², A. Dityatev², R. Gainetdinov²,
V. Broccoli¹

¹San Raffaele Scientific Institute, Milan, Italy

²Italian Institute of Technology (IIT), Genoa, Italy

Having access to human neurons for regenerative therapies and understanding diseases has been prohibited for long time. However, genetic technologies of cell reprogramming have wide opened this possibility by in vitro differentiation of fibroblast-derived iPS cells. However, a new method of cell reprogramming has become available which convert fibroblasts directly into functional neurons without passing through a pluripotent stem cell stage (iPSCs). This technology has some relevant advantages since it is very fast and efficient and can be employed with human adult fibroblasts. Recently, we found a minimal set of three transcription factors (Mash1, Nurr1 and Lmx1a) able to efficiently convert mouse and human fibroblasts into functional dopaminergic neuronal (iDAN) cells. Molecular and transcriptome studies showed iDAN cells to recapitulate gene expression of their brain homolog neurons to large extent while lacking expression of other monoaminergic and catecholaminergic neuronal subtype markers. Transgene expression is necessary for only 6 days to achieve a stable cell conversion sustained by activation of the endogenous genes of the three reprogramming factors. Strikingly, iDAN cells showed spontaneous electrical activity organized in regular spikes consistent with the pacemaker activity featured by brain DA neurons and produce and release dopamine. Furthermore, iDAN cells express D2 autoreceptors and their activity is regulated by the D2/3R agonist quinperole. The three factors were able to elicit DA neuronal conversion in prenatal or adult fibroblasts from healthy donors and Parkinson's disease patients. Mouse iDAN cells integrated in orthotopic brain side after transplantation in the forebrain structures and matured into functional neurons. Importantly, when transplanted in rats unilaterally lesioned with 6-OHDA, iDAN cells were able to rescue drug-induced locomotor impairment over long time. We have recently improved human iDAN cell reprogramming conditions in order to establish an efficient system even when starting from wayward human adult fibroblast cell lines.

Poster Abstracts

(presenting authors are shown underlined)

P1

UHRF1-dependent miRNAs modulate Dnmt3a expression in prostate transformed cells

F. Babbio¹, V. DelVescovo², F. LaMastra¹, M. Grasso², I. Castiglioni¹, M. Mandruzzato¹,
C. Pistore¹, E. Magnani¹, M.A. Denti², I.M. Bonapace¹

¹Dept Theoretical and Applied Sciences, Univ. of Insubria, Busto Arsizio VA, Italy

²CIBIO Center, Univ. of Trento, Trento, Italy

MicroRNAs are a class of small (21-22 nucleotides) non-coding RNAs that negatively control gene expression. It is being increasingly recognized that microRNAs constitute a class of gene modulators that play crucial functions in fundamental cellular processes and a growing body of evidence indicated their deregulated expression in human tumours, proposing microRNAs as novel oncogenes or tumour suppressors.

We have recently demonstrated that UHRF1 protein is an epigenetic “crosstalker” involved in prostate cancer progression and its depletion determines reactivation of numerous tumour-suppressor genes. In this study, we investigate whether this UHRF1 role could include changes in microRNAs levels in prostate transformed cells (PC-3).

Interestingly, microRNAs expression profile of siRNA-UHRF1 PC-3 cells showed both increased and decreased miRNAs levels.

Using different predictive softwares to identify putative microRNAs targets, Dnmt3a appears to be a putative target of two miRNAs, which are overexpressed in siRNA-UHRF1 PC3 cells.

Dnmt3a is a DNA de novo methyltransferase highly expressed in various malignancies, including prostate tumours. The overexpression of the two miRNAs was confirmed by RT-PCR in siRNA-UHRF1 compared to siRNA-CTRL PC-3 cells. We also observed a Dnmt3a protein reduction in UHRF1 depleted PC-3 cells. The direct binding of the two miRNAs to the 3'UTR of Dnmt3a and its potential inhibitory effect was functionally validated using a reporter luciferase system. We then studied the effect of the overexpression of the two miRNAs in PC-3 cells finding a decrease of Dnmt3a mRNA and protein.

Next goal in our project is to identify and study miRNA promoters, and evaluate a direct or indirect role of UHRF1 in miRNAs transcription.

P2

Comprehensive analyses of noncoding RNAs in Polycomb-mediated myogenic differentiation

M. Baniol¹, B. Bodega¹, P. Carninci², T. Ravasi³, V. Orlando¹

¹Dulbecco Telethon Institute at IRCCS Santa Lucia, Epigenetics and Genome Reprogramming lab, Rome, Italy

²Omics Science Center, RIKEN Yokohama Institute, Yokohama, Japan

³KAUST, Thuwal, Saudi Arabia

Epigenetic mechanisms play a major part in myogenesis. Polycomb group proteins (PcG) are epigenetic regulators able to maintain the repressive state of specific set of genes involved in cell fate determination by controlling chromatin structure. It is now established that they play a key role in the maintenance of the skeletal muscle progenitors identity and that their dynamic regulation is crucial for terminal muscle differentiation. Over the past few years, studies have also unraveled the ability of non-coding RNAs to directly interact with PcG proteins and to be necessary for gene silencing. Still, the real extent of non-coding RNAs contribution to Polycomb recruitment and function remains to be determined. Our project aims at identifying and characterizing non-coding RNAs involved in PcG-mediated gene regulation, and their specific role in the myogenic differentiation program. Using techniques relying on high-throughput technologies, such as CAGE, ChIP-seq and RIP-seq, we are generating a set of databases where dynamic changes in transcription profile, Polycomb binding sites and Polycomb-associated RNAs are analyzed genome-wide in C2C12 myoblasts and derived myotubes. The crosstalk between these different databases will eventually lead us to an integrated view of the molecular machineries that control the specific recruitment of PcG complexes on their chromatin target sites. Such a deeper understanding of both PcG and ncRNA functions in myogenesis regulation could be of great relevance to determine the molecular basis of muscle diseases in humans.

P3

Using chromosome conformation capture as an antibody-independent sensitive melanoma biomarker

E. Bastonini, L. Sanchez Del Campo Ferrer, C.R. Goding

Ludwig Institute for Cancer Research, University of Oxford, Headington, Oxford, United Kingdom

Despite some decades of research almost half of patients diagnosed with cancer die of the disease. The failure of approaches to cancer therapy reflects an underlying problem: cancer cell population heterogeneity. Without having a marker set that distinguishes between cancer sub-populations it is not possible to know which population is being targeted in any therapy. Although antibodies against differentiation-associated markers are used, in most cancers no markers for invasive stem-like populations have been identified. Since chromatin structure may be related to or independent from gene expression, chromatin signatures represent an alternative to antibody markers that are strictly dependent on expression. Chromosome Conformation Capture (3C) can detect early changes in epigenetic regulatory signatures at loci implicated in cancer. Malignant melanoma is a cancer known for its resistance to most therapies. The microphthalmia-associated transcription factor (MITF) is not only the master regulator determining the identity of the melanocyte lineage, but it also has a critical role in melanoma by regulating survival, differentiation, proliferation and invasiveness. In melanoma biopsies, MITF marks a distinct sub-population of melanoma cells providing an illustration of melanoma tumor heterogeneity. Hence, depending on its expression level, MITF can have pro- or anti-proliferative effects. We used 3C assays to understand whether chromatin organization at the MITF gene is controlled by genomic interactions in three dimensional space. Using human cells and blood samples from melanoma patients, we identified multiple 3C signatures associated with MITF that may represent both expression dependent and independent chromatin conformations. In this context the role of the p66 protein of the NURD complex is significant for MITF chromatin loops. Thus, we propose that a distinct chromatin architecture coordinated by p66/NURD complex accompanies transcriptional activation of MITF.

P4

The PDZ-ligand and SRC-homology type 3 domains of epidemic avian influenza virus NS1 protein modulate human SRC kinase activity during viral infection

L. Bavagnoli¹, W.G. Dundon², A. Garbelli¹, B. Zecchin², A. Milani², G. Parakkal¹, F. Baldanti³, S. Paolucci³, R. Volmer⁴, Y. Tu⁵, C. Wu⁵, I. Capua², G. Maga¹

¹Institute of Molecular Genetics National Research Council, Pavia, Italy

²World Organization for Animal Health, Food and Agriculture Organization and National Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

³Molecular Virology Unit, Virology and Microbiology, Fondazione Istituto Ricovero e Cura a Carattere Scientifico Policlinico S. Matteo, Pavia, Italy

⁴Université de Toulouse, Institut National Polytechnique, Ecole Nationale de Veterinaire, Unite` Mixte de Recherche 1225, Interactions Hotes-Agents Pathogènes, Toulouse, France

⁵Dept of Pathology, Univ. of Pittsburgh, Pittsburgh, Pennsylvania, United States of America

The Non-structural 1 (NS1) protein of avian influenza (AI) viruses is important for pathogenicity. NS1 is not a structural component of the virion, but it is expressed at very high levels in infected cells. NS1 inhibits host immune response limiting both interferon (IFN) production and the antiviral effects of IFN-induced proteins. The last six aminoacids (225-230) of NS1, in strains of avian origin, constitute a PDZ-binding domain (PL), which allows interaction with a wide range of cellular proteins. We have identified in the last 12 amino acids of the C-terminal region of AI H7N1 NS1 a previously unreported rearrangement constituted by two consecutive PL motifs (TPL, Tandem PDZ-Ligand). Using a protein array, we have identified the cellular protein RIL (Reversion Induced-LIM) as the primary ligand protein of NS1. It is known that RIL interacts with the active form of tyrosine kinase Src, playing a leading role in its regulation. The avian NS1 proteins also contain two Src-homology type 3 binding domain (SHB), thus we tested its ability to physically and functionally interact with Src, both in the presence or absence of RIL, through enzymatic and pull down assays. The results show that NS1 is able to physically interact with Src, and to stimulate its enzymatic activity. In infected cells, the levels of phosphorylated (active) Src correlate with the ability of NS1 to bind RIL through its TPL domain, suggesting an interplay among these three proteins. However, the exact role of Src activation in the avian influenza virus life cycle is still unknown. Bioinformatic analysis of the human proteome, identified several potential cellular ligands for avian NS1. Due to its potential binding to multiple partners, NS1 can interfere with different cellular pathways. Our findings are aimed at defining a therapeutic strategy that can reduce the emergence of viral resistance towards antiviral drugs, based on the selective targeting of host factors required for viral replication.

P5

Epigenetic regulation of HBV cccDNA

L. Belloni^{1,2,3}, L. Allweiss⁴, F. Guerrieri^{1,2,3}, N. Pediconi^{1,2,3}, T. Volz⁴, T. Pollicino⁵, J. Petersen⁶, G. Raimondo⁵, M. Dandri⁴, M. Levrero^{1,2,3}

¹EAL Inserm U785, Sapienza University of Rome, Rome, Italy

²Laboratory of Gene Expression, Fond. A. Cesalpino, Rome, Italy

³Dept of Internal Medicine, Univ. La Sapienza, Rome, Italy

⁴Dept of Internal Medicine, Univ. Medical Hospital Hamburg-Eppendorf, Hamburg, Germany

⁵Dept of Internal Medicine, Univ. of Messina, Messina, Italy

⁶IFI Institute for Interdisciplinary Medicine at Asklepios Clinic St. Georg, Hamburg, Germany

The HBV cccDNA is the nuclear replicative intermediate of HBV and is the template for all viral messenger RNAs. It is organized into minichromosomes in the nucleus of infected cells by histone and non-histone proteins and its function is regulated, similarly to cellular chromatin, by the activity of nuclear transcription factors, transcriptional coactivators and corepressors and chromatin modifying enzymes. We developed a chromatin immunoprecipitation (ChIP)-based quantitative technique that allows to define the enzymatic activities that bind to and regulate transcription/replication from the cccDNA minichromosome. We previously showed that HBV replication is regulated, both in a cell replication system and in the liver of HBV chronically infected patients, by the acetylation status of cccDNA-bound H3/H4 histones.

Interferon-alpha (IFN α) is an effective treatment for hepatitis B virus (HBV) infection. We observe that IFN α exerted an inhibitory effect on HBV replication. Inhibition of replication was paralleled by a very strong decrease in pgRNA transcription without observing significant changes in the levels of cccDNA. Using a cccDNA specific chromatin immunoprecipitation assay we found that, in response to IFN α , cccDNA-bound histones are hypoacetylated and transcriptional co-repressors HDAC1, hSIRT1 and Ezh2 are actively recruited onto the cccDNA. Moreover, we also show that both STAT1 and STAT2 transcription factors are recruited onto the cccDNA and their binding is reduced after IFN α treatment. An ISRE mutant HBV doesn't have STAT2 bound to the cccDNA, transcribes less viral RNA, cannot be repressed by IFN α and fails to recruit HDAC1 in response to IFN α . Altogether our results indicate that IFN α can affect the transcriptional activity of the cccDNA epigenetically and provide a molecular mechanism for IFN α repression of HBV transcription.

P6

Characterisation of a novel long non coding RNA potentially involved in neuronal differentiation

V. Bevilacqua¹, U. Gioia¹, I. Bozzoni^{1,2,3}, E. Caffarelli^{2,3}

¹Dept of Biology and Biotechnology, Sapienza Univ. of Rome, Italy

²Institute of Molecular Biology and Pathology-CNR, Rome, Italy

³Center for Life Nano Science, Istituto Italiano di Tecnologia, Sapienza Univ. of Rome, Italy

Recent genome-wide analyses have shown that mammalian genomes are pervasively transcribed and encode many thousands of non coding RNAs (ncRNAs) that differ for size, biogenesis and function. Notably, a large number of these transcripts exert regulatory functions, contributing to the complexity of higher eukaryotes. Long non coding RNAs (lncRNAs), greater than 200 nucleotides in length, are one of the most abundant class of ncRNAs transcribed in a developmentally regulated and cell-specific manner. Despite their abundance, only a small fraction of lncRNAs has been associated to biological functions or disease processes. An ideal model system for studying the relevance of such molecules is the nervous system, whose development is a highly regulated process orchestrated by several different regulatory mechanisms acting at both the transcriptional and post-transcriptional level. In line with such complexity, it has been shown that a half of all lncRNAs is expressed in the central nervous system (Quereshi et al., 2010).

Starting from our studies aimed at unveiling microRNAs (miRNAs), a class of short ncRNAs with regulatory functions, involved in neuronal differentiation we identified a lncRNA in which miR-125b-1 coding region is embedded (linc125b-1). As the miRNA, linc125b-1 is upregulated during *in vitro* differentiation of human neuroblastoma cells, suggesting that the expression of the two non coding RNAs is co-regulated during the differentiation process. Notably, linc125b-1, that has a predominant cytoplasmic localization, displays several putative binding sites for miRNAs known to counteract cellular differentiation. Functional studies are in progress to verify its potential role as competing endogenous RNA (ceRNA), which may be directly implicated in the choice of neuronal differentiation programs by sequestering specific miRNAs that inhibit such process (Poliseno et al., 2010; Cesana et al., 2011).

P7

Italian strains of *Babesia bovis*: first description of surface antigens and molecular markers

A. Torina¹, V. Blanda^{1,2}, A. Agnone², V. Di Marco¹, S. Caracappa¹, S. Brigano¹, I. Albanese², S.E. Wilkowsky³, M. Florin Christensen³

¹Istituto Zooprofilattico Sperimentale della Sicilia

²Univ. degli Studi di Palermo

³Biotechnology Institute INTA, Castelar, Argentina

Babesiosis is a tick-borne disease mainly transmitted by the tick *Rhipicephalus* spp. Among bovine parasites, *Babesia bovis* is one of the most relevant pathogens, causing in its hosts a severe anaemia, high fever and, sometimes, the death. Many efforts are directed to the study of *B. bovis* surface antigens as vaccine candidates, as well as to the finding of new molecular markers to track the strains provenience.

Two Italian strains of *B. bovis* were analysed to amplify genes codifying for the Merozoite Surface Antigen 2c (MSA-2c) and for the Apical Membrane Antigen-1 (AMA-1), two proteins deeply studied thanks to their possibility to be included in a subunit vaccine. The two sequences were amplified in the Italian strains, cloned and sequenced and the data obtained were compared with those available in GenBank. Both MSA-2c and AMA-1 showed at aminoacidic level a very high percentage of identity each other (93.0 % for MSA-2c and 99.4% for AMA-1) and with the sequences annotated in GenBank (an average of 93.8% for MSA-2c and of 97.2% for AMA-1). Furthermore, six B-cell epitopes were identified in the MSA-2c aminoacidic sequences by bioinformatics. Out of these, two were entirely conserved among Italian and geographically distant strains.

Genes recently identified suitable for molecular markers, *Desmoyokin* and *85KDa*, were also amplified and sequenced. The analysis allowed defining the repeats pattern of Italian strains. Interestingly, it was noted a conservation in the *consensus* pattern, but Italian strains showed a pattern never found in foreign strains.

The obtained results constitute the first information related to Italian strains of *Babesia bovis* and reinforce the hypothesis to use MSA-2c and AMA-1 proteins as vaccine candidates and *Desmoyokin* and *85KDa* genes as molecular markers.

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P8

UbcD1: from telomere capping to global heterochromatin regulation

G. Burgio^{1,2}, D. Corona^{1,2}

¹Dulbecco Telethon Institute c/o ² Dept STEMBIO , Univ. of Palermo, Palermo, Italy

Eukaryotic cells store their genetic information into chromatin, in which genomic DNA is associated with histone and non histone proteins, forming an highly dynamic structure involved in the control of numerous biological processes, including transcription, cell division, differentiation and DNA repair. Chromatin plasticity is the result of the combined action of ATP-dependent chromatin remodelling activities and histone modifying enzymes, which modulate the epigenetic marks of eukaryote genome. In contrast to the well-characterized histone modifications, such as acetylation and methylation, the role of histone ubiquitination in chromatin remodelling remains the least understood despite a long history of its discovery. We found that *effete*, the gene encoding for the putative ubiquitin E2 conjugating enzyme UbcD1, genetically interacts with the ATP-dependent chromatin remodeler encoding gene *ISWI*, suggesting a potential role of UbcD1 in chromatin modulation. The analysis of *Drosophila* *effete* knock-down polytene chromosomes reveals not only chromatin condensation defects, highly reminiscent of those caused by loss of *ISWI*, but also a dramatic enhancement of those defects when the knock-down is induced in *ISWI* genetic background. Moreover *effete* loss of function causes a clear decrease of chromosomal mono-ubiquitylated H2B level. Interestingly we found that overexpression of *osa*, a recently identified E3 ubiquitin ligase specific for H2B, partially suppresses both chromosomes condensation defects and lethality induced by *effete* knock-down, suggesting that mono-ubiquitylated H2B level could be directly linked to chromosomes condensation levels. Moreover, loss of UbcD1 induces global loss of the linker histone H1, suggesting the intriguing possibility that UbcD1 and its ubiquitylating activity could be among the factors regulating higher order chromatin structure.

P9

TRIM8: a new key modulator of the p53 pathway

M.F. Caratozzolo¹, F. Mastropasqua², I. Aiello¹, T. Lopardo³, F. Marzano¹, A. Valletti⁴,
A.M. D'Erchia², G. Pesole^{2,4}, E. Sbisà¹, L. Guerrini³, A. Tullo¹

¹Istituto Tecnologie Biomediche ITB, CNR-Bari, Bari, Italy

²Dipartimento di Bioscienze, Biotecnologie e Scienze Farmacologiche, Università degli Studi di Bari "A. Moro", Bari, Italy

³Dipartimento di Science Biomolecolari e Biotecnologie, Univ. di Milano, Milano, Italy

⁴Istituto Biomembrane e Bioenergetica IBBE, CNR-Bari, Bari, Italy

p53 regulation occurs through a vast number of mechanisms and the inactivation of its pathway is a common feature of human cancer.

We found that human TRIM8, a member of the TRIM family, is a new modulator of the p53-mediated tumour suppression mechanism. Under genotoxic stress, p53 induced the expression of TRIM8, which in turn stabilized p53 and promoted the degradation of MDM2, the principal regulator of p53 stability. Co-immunoprecipitation experiments showed that TRIM8 physically interacted with p53, impairing its interaction with MDM2 (Caratozzolo MF et al., 2012).

TA and ΔN isoforms of the p53 family members display a fine and very complex interplay between them. Therefore, we investigated the effect of TRIM8 on the oncogenic $\Delta Np63\alpha$, the main p63 isoform involved in cancer development. We found that TRIM8 overexpression induced degradation of $\Delta Np63\alpha$ in a dose dependent manner, while its silencing resulted in a pronounced accumulation of endogenous $\Delta Np63\alpha$ accompanied by a decrease of p53 levels in parallel with an increase in cell proliferation.

Interestingly, only the TRIM8 mutant protein lacking the RING domain was unable to suppress cell proliferation, to promote stabilization of p53 and degradation of both MDM2 and $\Delta Np63\alpha$. Thus, we tested whether TRIM8-RING domain alone was able to enhance p53 activity. Indeed, overexpression of the TRIM8-RING domain induced cell cycle arrest in a p53-dependent manner.

Altogether, our results reveal a new regulatory pathway controlling p53 and $\Delta Np63\alpha$ activities and suggest TRIM8 as a novel therapeutic target to simultaneously enhance p53 oncosuppressor and impair $\Delta Np63\alpha$ oncogenic activities.

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P10

Identification of GABA receptor genes and evidence of GABA signaling during embryogenesis of the sea urchin

S. Casamirra, G. Spinelli, V. Cavalieri

Dept Molecular and Biomolecular Sciences and Technologies (STEMBIO), Univ. of Palermo, Italy

Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian central nervous system and acts via ionotropic ($GABA_A$ -R_s) and metabotropic ($GABA_B$ -R_s) receptors. $GABA_A$ -R_s are Cl⁻ selective hetero-pentameric channels assembled by combinations of 19 distinct gene products. Instead, $GABA_B$ -R_s are bi-subunit G-protein coupled receptors linked to K⁺ or Ca²⁺ channels. Dysfunctions of GABA-signaling (GS) cause psychotic disorders and correlate with epigenetic alterations, such as over-expression of DNA methyl transferase-1 which in turn imposes iper-methylation of GABA-regulated genes. The sea urchin embryo, which presents a rudimentary nervous system, offers a big opportunity to study the GS and its potential epigenetic implications in a simple eukaryote model. To this purpose, we performed a comprehensive *in silico* analysis of the sea urchin genome and identified a 450 Kb long cluster containing two genes encoding for the $GABA_B$ -R subunits, and two genes respectively encoding for a $\alpha/\gamma/\epsilon$ -type and a $\beta/\rho/\delta/\tau/\pi$ -type $GABA_A$ -R subunit. From an evolutionary perspective, this result revealed a unique genomic organization of these genes in sea urchin. Next, to preliminarily evaluate the role of GS during development, *Paracentrotus lividus* embryos were cultured in the presence of GABA at concentrations ranging from 0.01 to 1.0 mM. Compared to controls, treated embryos showed aberrations in axial patterning, with a dose-dependent effect. In particular, at 48 hours post-fertilization control embryos were normal bilateral symmetric plutei whereas GABA-treated embryos displayed a radial organization with supranumerary spicules. Washout experiments allowed to determine that the period of sensitivity is restricted from the blastula to the gastrula stage. Altogether, these results suggest that dysregulation of GS affects the polarization of the ectoderm. Although preliminary, this study provide the first evidence of GS activity during development of echinoderms.

P11

Relationship between autophagy and apoptosis in *Paracentrotus lividus* embryos cadmium exposed

R. Chiarelli, M. Agnello, G. Morici, M.C. Roccheri

Dept STEMBIO (Biologia Cellulare) Univ. of Palermo, Italy

Cadmium is a strong toxicant for living organism since it does not have biological roles and can cause several cellular damages comprising genotoxicity, oxidative stress, and other biochemical dysfunctions. Marine invertebrate embryos represent a suitable model system where to investigate the effects of many stressors on development and cell viability. Here we investigated the toxic effect of cadmium on sea urchin *Paracentrotus lividus* embryos focusing our attention on metal-induced autophagy and the possible temporal and functional relationship with apoptosis.

Using several techniques to detect autophagy (neutral red, acridine orange and LC3-detection) we demonstrated that Cd-exposed *P. lividus* embryos adopt this process as an additional stratagem to safeguard the developmental program. In particular we observed that embryos treated at sublethal Cd concentration activate a massive autophagic response after 18h, which decreases between 21 and 24h, in the opposite of apoptotic process.

In order to investigate a possible temporal relationship between autophagy and apoptosis, we tested apoptotic signals by TUNEL and immunofluorescence *in situ* assays of cleaved caspase-3. Quantitative analysis has shown that embryos activate a massive apoptosis after 24h of Cd-exposure. Therefore a functional relationship between autophagy and apoptosis was estimated evaluating apoptotic signals in Cd-exposed embryos, upon treatment with the autophagic inhibitor 3-methyladenine. We found that the inhibition of autophagy produced a contemporaneous reduction of apoptotic signals, suggesting that the two phenomena are functionally related. In effect using methylpyruvate, a cell-permeable substrate for ATP production, apoptotic signals were substantially restored.

These data could be explained considering that autophagy could energetically contribute to apoptotic execution through its catabolic role.

P12

LSD2: A new flavin-containing histone demethylase

G. Ciossani, A. Karytinou, M. Foglizzo, C. Binda, A. Mattevi

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

For long time the methylation of histone lysines was considered an irreversible epigenetic modification, but the discovery of the first Lysine Specific Demethylase (LSD1), showed the dynamic regulation of this mark.

LSD1, in complex with the corepressor CoREST and recruited by different multiprotein complexes, is involved in many biological processes like cell differentiation and development. In addition to the LSD1 gene (aof2), mammalian genomes contain a second gene (aof1) coding for a protein, named LSD2, that is 33% identical to LSD1. Despite this similarity, LSD2 differs from LSD1 in that it contains a N-terminal CW-type zinc finger and lacks the Tower domain, the domain responsible for the interaction with CoREST. Our studies demonstrated that also LSD2 is a flavin-dependent lysine-specific histone demethylase, biochemically very similar to LSD1. Differently from LSD1, this protein does not interact with CoREST and the class 1 Histone Deacetylases (HDACs) 1 and 2. Taken together, these data indicate that LSD2 belongs to macromolecular complexes distinct from those of LSD1, suggesting a completely different biological role. A little is known about the biology of LSD2: In mice, it is mainly expressed in intestinal glands and during late oogenesis, when its activity is fundamental for the correct establishment of DNA methylation patterns.

The aim of my PhD project is the structural and biochemical characterization of LSD2. So far, the efforts to crystallize the single domains or the entire LSD2 protein, from both human and mouse, gave no results.

P13

Role of ISWI chromatin remodeler in epigenetic reprogramming occurring in regeneration and transdetermination

G. D'Angelo^{1,2}, D.F.V Corona^{1,2}

¹Dulbecco Telethon Institute c/o ²Dept STEMBIO, Palermo Univ., Palermo, Italy

Epigenetic modifications of chromatin structure sustain cell fate determination over multiple mitotic divisions throughout development, setting the epigenetic cell memory, thus the cell identity. Several evidences demonstrated that in all metazoan, chromatin modifications are reversible also in terminally differentiated cells. During regeneration, a well evolutionarily conserved phenomenon, yet determined cell do not only proliferate but extensively reprogram their epigenetic memory to regenerate missing parts of injured tissue. Moreover, during regeneration, the plasticity of chromatin structure allows cells to switch cell identity thus to trans-determine their cell fate.

Accumulating evidences suggest a crucial role of chromatin remodelers in epigenetic reprogramming occurring in regeneration and transdetermination, still molecular mechanisms underlying these processes are poorly understood. *D. melanogaster* larval imaginal discs, the precursor of fly appendages, provide a unique system to investigate about the potential of cell to regenerate and to transdetermine.

Our aim is to investigate the role of the ATPase-dependent chromatin remodeler ISWI and its previously identified genetic modifiers in epigenetic reprogramming events occurring during regeneration and transdetermination.

We are employing the heat inducible “flp-out” method to generate fly stocks carrying clones of imaginal disc cells with high transdetermining or high regenerating potential. We will compare the transdetermination and the regeneration rates of this induced clones to ISWI modifiers yet identified in our lab, in order to identify genes enhancing or suppressing epigenetic reprogramming.

Our study will hopefully provide crucial insights into the roles that ATP-dependent chromatin remodeling reactions may play in transdetermination and into the ability to control tissue regeneration.

P14

A role for the lysine-specific demethylase 1 (LSD1) in hepatic stem cell fate determination

C. Cicchini¹, V. De Nonno¹, L. Amicone¹, S. Valente², M. Tardugno², A. Mai², M. Tripodi¹

¹Istituto Pasteur-Fondazione Cenci Bolognetti, Dept of Cellular Biotechnologies and Haematology, Univ. Sapienza of Rome

²Dept of Drug Chemistry and Technologies, Univ. Sapienza of Rome

A number of hepatic stem cell lines named RLSCs (from Resident Liver Stem Cells) were previously stabilized and characterized in our lab (Conigliaro et al., 2008). These cells spontaneously differentiate into periportal hepatocytes that, in turn, are able to switch into perivenular hepatocytes (Colletti et al., 2009). RLSCs show a metastable molecular phenotype typical of embryonic and adult stem cells and characterized by co-expression of epithelial and mesenchymal markers as well as of a variety of chromatin remodeling genes. This highly dynamic cell state, thought to be the major determinant of stem cell fate, may be considered as the result of continue oscillations between Epithelial-to-Mesenchymal Transition (EMT) and the reverse Mesenchymal-to-Epithelial Transition (MET).

Taking into account the recently reported role for the lysine-specific demethylase1 (LSD1) in triggering EMT, we hypothesized that a bulk changes in histone modifications might underlie the EMT/MET dynamics in the metastable molecular phenotype of the hepatic stem cells, influencing their differentiation potential.

By using of specific chemical inhibitors, we unveiled a role for LSD1 in influencing the RLSC plasticity.

P15

FAIRE and 3C analysis of chromatin modifications induced by the HNF1 β transcription factor in the albumin/alpha-foetoprotein (alb/AFP)

M. Olivieri, G. D'Agostino, A. Ingenito, V. De Pasquale, L. De Magistris, V. De Simone
Dept of Biochemistry and Medical Biotechnology, Univ. "Federico II", Napoli, Italy

AIMS OF THE PROJECT: To investigate the role of the HNF1 β transcription factor in the commitment of epithelial cells to the hepatic fate by inducing modifications of the chromatin in a complex locus containing two liver-specific genes.

SCIENTIFIC BACKGROUND: Hepatic Nuclear Factor 1 beta (HNF1 β) transcription factor is expressed very early during development, approximately when the hepatic fate and, differently from its cognate HNF1 α , largely before any liver-specific gene is expressed. These two factors bind the same DNA target sequences and their protein sequences are very similar in the DNA binding domain, but remarkably different in the C-terminal domain. Our working hypothesis is that HNF1 β acts mainly as a chromatin modifier, by increasing the accessibility of HNF1 α and other liver-specific factors to the main regulatory elements of liver-specific genes. The albumin/alpha-foetoprotein (alb/AFP) locus is a good candidate for chromatin modification analysis, since it contains several regulatory elements scattered in a 40 kb region. Moreover, the locus is involved into a developmentally regulated foetal/adult transcriptional switch.

METHODOLOGY: Chromatin extracted from hepatic and non-hepatic human and mouse cell lines transfected with expression vectors containing the HNF1 β coding region have been analysed by FAIRE (Formaldehyde Assisted Isolation of Regulatory Elements) and 3C (Chromosome Conformation Change) techniques to reveal modifications of the accessibility and/or the formation of loops between distant regulatory elements.

RESULTS: Our preliminary results indicate that transfection of HNF1 β in non-hepatic cells increases the accessibility of albumin and AFP promoters, while HNF1 α or a truncated form of HNF1 β (deprived of the C-terminal region) do not induce any chromatin modification. 3C experiments in both hepatic and non-hepatic cell lines are currently in progress.

P16

LINE1 copy number changes during transdifferentiation of MEF into iDA neurons

F. Della Valle¹, M. Caiazzo², B. Bodega¹, V. Broccoli², V. Orlando¹

¹Dulbecco Telethon Institute at IRCCS Fondazione S.Lucia, Rome

²San Raffaele Scientific Institute, Division of Neuroscience, Milan

LINE1 class of repetitive elements is composed by sequences capable of active retrotransposition and by elements carrying deletion in the 5' UTR or mutation inside the ORF sequence that stuck those one in their position. (Moutri et al.2005).Some papers in the latest years showed that repetitive elements can be very important for the establishment of a gene expression profiles. It is also recently demonstrated that full-length LINE1 mRNA is expressed in iPSC and an engineered LINE1 can retrotranspose much better in iPSC than in HDF, because reprogramming reinstates LINE1 transcription and creates the environment required for their reintegration in the genome (Wissing et al.2011). It seems reasonable to hypothesize that If L1 retrotransposition activity is fundamental for a physiological gene expression pattern; it could be important to remobilize these repetitive sequences during transdifferentiation. This may allow the remodeling of the chromatin required for the switch of gene expression profile. The transdifferentiation of embryo fibroblast into induced dopaminergic neurons(iDA), by the overexpression of three neural transcription factors and using a neural culture media(Caiazzo et al.2011), represents a suitable model for studying the involvement of LINE1 in the cell fate decision. We already analyzed L1 copy number during myogenic differentiation and we observed a different copy number patient of Duchenne muscular dystrophy respect to healthy donors. For those reason we begin the analysis of full length L1 elements transcription and copy number variation during transdifferentiation process whit a specific taqman assay showed a gradual increase of LINE1 expression and number of copy of those elements. Taken together, those data may effort the idea that LINE1 can be remobilized under a specific signaling pathway when the cell fate is established during differentiation. Moreover remobilization of this class of repetitive element may be crucial to obtain a more efficient reprogramming protocol.

P17

Mechanisms of altered transcriptional regulation in the pathogenesis of AEC syndrome

G. Ferone^{1,2}, A. Sirico¹, C. Missero^{1,2}

¹CEINGE Biotecnologie Avanzate, Napoli, Italy

²Fondazione IRCCS SDN, Napoli, Italy

The p53 homologue p63 is a tetrameric transcription factor with a pivotal role in stratified epithelial development and maintenance. Heterozygous mutations in p63 cause at least five closely-related inherited disorders, among which Ankyloblepharon-Ectodermal dysplasia-Clefting (AEC) syndrome is characterized by severe involvement of the skin. To elucidate the molecular mechanisms underlying this disorder, we previously generated a knock-in mouse model (p63^{+/L514F}) carrying a clinically relevant missense mutation in the carboxyl-terminal region of the p63 α isoform, in which AEC mutations are clustered. p63^{+/L514F} mice are affected by cleft palate, skin hypoplasia and ectodermal dysplasia, thus faithfully recapitulating the phenotype observed in AEC syndrome. Cleft palate and skin hypoplasia are caused by hypoproliferation of epithelial progenitors and reduced number of epidermal stem cells associated with downregulation of FGF signalling and FGFR2 expression.

Mechanistically we find that mutant p63L514F protein retains the ability to form tetramers with itself and the wild-type protein, however the tetramer has an impaired transactivation activity. In mutant cells reduced transactivation is associated with partially impaired p63 binding to DNA in the chromatin context and a modest impairment of tri- and di-methylation of histone H3 at lysine 4 at the p63 binding sites. Consistently, in the absence of the wild-type protein, p63L514F is severely impaired in its ability to bind DNA in the genomic context and is unable to transactivate its target genes. These findings indicate that mutant p63 acts in a dominant-negative fashion affecting p63 ability to transactivate crucial targets in epidermal development.

P18

Molecular characterization of the RNA binding motif protein 20: determination of nuclear localization signals

A. Filippello, P. Lorenzi, C. Morandi, M.G. Romanelli

Dept of Life and Reproduction Sciences, Section of Biology and Genetics, Verona, Italy

RNA splicing is a tightly regulated process that involves the spliceosome and additional RNA binding proteins that can repress or activate splice sites selection. Recent studies have indicated that mutations in RBM20, a gene encoding a novel ribonucleic acid – binding protein, are associated to human dilated cardiomyopathy (DCM). RBM20 regulates alternative splicing of expressed genes that have a key role in cardiac function, including ion homeostasis, sarcomere biology, and signal transduction. The functional motifs of the RBM20 protein, have been poorly investigated. The focus of this study is to characterize the protein domains that contribute to the nuclear function of RBM20. Predictive in silico analysis of the translated RBM20 gene identifies an RNA recognition motif (RRM motif), a serine /arginine (RS) domain and Zn²⁺ finger domains. We have produced GFP-RBM20 fusion proteins in order to map the functional domains of the protein that contribute to subcellular distribution. We have produced truncated mutants of the RBM20 proteins and analyzed separately in immunofluorescence assays in transfected cells. We identified a region necessary and sufficient to nuclear localization of RBM20 protein that maps between the RRM and the RS domain. Actually we are producing RBM20 mutant proteins in order to characterize the nuclear localization signal (NLS). RBM20 is the first RNA binding protein associated to DCM. Further structural and functional characterization of RBM20 may contribute to understand the molecular pathogenesis of familiar DCM.

P19

Functional complementation of yeast *sir2Δ* mutation by the human orthologous gene SIRT1

D. Gaglio, E. Cesarini, A. D'alfonso, G. Camilloni

Dept of Biology and Biotechnology "Charles Darwin", Univ. of Rome SAPIENZA, Rome, Italy

Sirtuins (SIRT1-7), or class III histone deacetylases (HDACs), are deacetylases/ADP-ribosyltransferases that target a wide range of cellular proteins in the nucleus, cytoplasm, and mitochondria for post-translational modification by deacetylation (SIRT1, -2, -3 and -5) or ADP-ribosylation (SIRT4 and -6). The orthologs of Sirtuins in lower organisms play a critical role in regulating lifespan, aging and metabolic diseases. The mammalian Sirtuins SIRT1-SIRT7 are implicated in a variety of cellular functions ranging from gene silencing, over the control of cell cycle and apoptosis, to energy homeostasis. On a whole-body level, Sirtuins display a wide range of activities in the cell, which suggests that they may constitute therapeutic targets to contrast metabolic, neurodegenerative (Parkinson and Alzheimer), and proliferative diseases such as cancer.

Specifically, this project is about the molecular cloning of the human sirtuin member SIRT1 in *Saccharomyces cerevisiae*. We cloned this enzyme into an engineered yeast plasmid with a galactose inducible promoter and we used it for heterologous expression in *S. cerevisiae* mutants for the ortholog SIR2. To verify total or partial complementation, after having established that SIRT1 has no toxic effect on yeast, we studied their behaviour by analyzing different molecular phenotypes such as: i) the transcriptional repression in rDNA, HM loci and telomeres; ii) histone deacetylation (H4K16) and iii) the production of extrachromosomal rDNA circles (ERCs). The aim of this work is to determine whether human gene complementation occurs in *Saccharomyces cerevisiae* in order to use yeast as a model organism to conduct molecular studies on diseases where this enzyme is involved, e.g., Parkinson's, Alzheimer's, prostate cancer and diabetes. A more specific use of this complemented yeast strain is the screening of activator and inhibitor molecules of Sirtuins for a therapeutic approach.

P20

ADAR2 editing activity inhibits glioblastoma growth through the modulation of the CDC14B/Skp2/p21/p27 axis

F. Galeano¹, S. Tomaselli¹, L. Cifaldi¹, L. Massimi², F. Locatelli^{1,3}, A. Gallo¹

¹Oncohaematology Dept, IRCCS Ospedale Pediatrico “Bambino Gesù”, Rome, Italy

²Policlinico Gemelli, Pediatric Neurosurgery Dept, Rome, Italy

³Univ. di Pavia, Pavia, Italy

Grade IV astrocytoma or glioblastoma multiforme (GBM) is one of the most aggressive and lethal tumors affecting humans. ADAR2-mediated A-to-I RNA editing, an essential post-transcriptional modification event in brain, is impaired in GBMs and astrocytoma cell lines. However, the role played by ADAR2 editing in astrocytomas remains to be defined.

Here, we show that ADAR2 editing rescue in astrocytomas prevents tumor growth *in vivo* and modulates an important cell-cycle pathway involving the Skp2/p21/p27 proteins, often altered in glioblastoma. We demonstrate that ADAR2 deaminase activity is essential to inhibit tumor growth. Indeed, we identify the phosphatase CDC14B, which acts upstream of the Skp2/p21/p27 pathway, as a novel and critical ADAR2-target gene involved in glioblastoma growth.

Specifically, ADAR2-mediated editing on CDC14B pre-mRNA increases its expression with a consequent reduction of the Skp2 target protein, as shown both *in vitro* and *in vivo*. We found that, compared to normal brain, both CDC14B editing and expression are progressively impaired in astrocytomas from grade I to grade IV, being very low in GBMs.

These findings 1) demonstrate that post-transcriptional A-to-I RNA editing might be crucial for glioblastoma pathogenesis, 2) identify ADAR2 editing enzyme as a novel candidate tumor-suppressor gene and 3) provide proof of principle that ADAR2 or its substrates may represent a suitable target(s) for possible novel, more effective and less toxic approaches to the treatment of GBMs.

P21

Genome-wide analysis of the repertoire of TRIM genes in sea urchins

R. Guarcello, G. Spinelli, V. Cavalieri

Dept Molecular and Biomolecular Sciences and Technologies (STEMBIO), Univ. of Palermo, Italy

The eukaryotic TRIM (TRIPartite Motif) super-family represents one of the largest classes of putative E3 ubiquitin ligases involved in several processes, including epigenetic control of development and disease. In the post-genomic era, new approaches allow genome-wide studies of gene family. In particular, we performed a comprehensive analysis of the TRIM repertoire in selected sea urchin species. By combining iterations of *ab initio* predictions and pairwise comparative methods, we first retrieved the full complement of TRIM genes in *Strongylocentrotus purpuratus*, whose full genome sequence was available. Interestingly, such a DNA sequence set includes not previously classified, echinoderm-specific, TRIM genes as well as multiple copies of known ones. We also retrieved a landscape of cDNA sequences from staged EST libraries, indicating that most of these genes are actively transcribed during development. Phylogenetic analysis of the deduced proteins, using set of TRIMs from various species, revealed a degree of genetic variation between species. Worth of mention, we predicted the occurrence of transposition events involving some of these genes, according with the documented rapid evolution of this family. Next, we adopted heuristic algorithms and post-processing steps to investigate the evolutionarily distant *Paracentrotus lividus*, *Allocentrotus fragilis* and *Lytechinus variegatus* genomes, whose sequencing projects are actually in progress. We assembled partial pools of TRIM genes and specifically associated them to EST-derived cDNA sequences. Such a collection of data should provide a framework for unravel gene regulatory networks involving TRIM genes from an evolutionary perspective. Indeed, in the *Pl* species, we have previously isolated and functionally characterized the cDNA sequence encoding the first echinoderm TRIM factor, Strim1. Here, we identified five *strim1* genes, all sharing a intronless organization, and roughly located their *cis*-regulatory apparatus.

P22

A functional loop involving the viral protein HBx and miR224 regulates HBV transcription and replication

F. Guerrieri, C. Scisciani, L. Belloni, A. Sbardellati, D. D'Andrea, A. Tramontano, M. Levrero, N. Pediconi

Dept of Internal Medicine, DMISM, Sapienza Univ., Rome, Italy, Rome, Italy

miR224 is frequently up-regulated in HBV and HCV-related HCCs, whereas its levels are low in acute HBV hepatitis. miR224 expression in the liver has been shown to be induced by the NFkB-dependent inflammatory pathways. We sought to characterize the transcriptional regulation of miR224 in HBV replicating cells, the role of HBx and miR224 effects on HBV replication. The HBV replicative intermediate cccDNA is the template for transcription of all viral mRNAs, and it accumulates in the nucleus of infected cells as a stable minichromosome. We have recently shown that HBV transcription and replication are regulated by epigenetic changes of cccDNA-bound histones mediated by the recruitment of both cellular chromatin modifying enzymes and the HBx viral regulatory protein. HBx has been also shown to bind the promoter regions of cellular genes. In a genome wide search of HBx cellular targets by ChIP-Seq we found that HBx binds in vivo to the miR224 regulatory region. HBx binding is accompanied by the co-recruitment of p65/NFkB, the recruitment of the DNMT3a and reduced H4 histone acetylation. Accordingly, miR224 levels are reduced in HBV replicating cells and exogenously expressed HBx repressed the miR224(pr) in luciferase reporter assays. pre-miR224 overexpression reduces HBV pgRNA levels and leads to a 50% inhibition of HBV replication. In silico analysis revealed the presence of several miR224 seed sequences on the HBV genome that were conserved across HBV genotypes. The functional analysis of miR-224 HBV seed sequences shows that multiple pgRNA seeds are targeted by mir224. Our results identify a functional regulatory loop between HBx, miR224 and HBV replication where HBx repression of miR224 expression relieves the negative effects of miR224 on HBV replication. These results are compatible with the recent observation that miR224 are low in acute HBV infection as compared to chronic infection and HCC and with the low HBV replication observed in HCC tissues.

P23

MicroRNA regulation by mutant p53 oncoprotein

F. Garibaldi, G. Bossi, A. Sacchi, G. Piaggio, A. Gurtner

Experimental Oncology Dept, Istituto Regina Elena, Rome, Italy

Deregulated miRNA expression has been documented in diverse cancers. Although miRNAs can function as both tumour suppressors and oncogenes in tumour development, a widespread downregulation of miRNAs is commonly observed in human cancers and promotes cellular transformation and tumorigenesis. Still, the mechanisms through which miRNAs are regulated in cancer remain unclear. Wtp53 is a key tumor suppressor mutated in approximately 50% of human cancers. Mutp53 proteins can acquire GOF activities favoring tumor induction, maintenance, spreading. miRNAs can be regulated by wtp53 at transcriptional level but data about mutp53 dependent miRNA expression are not available yet. wtp53 interaction with the Drosha processing complex facilitates the processing of pri-miRNAs to pre-miRNAs. On the contrary, an overexpressed mutp53 disrupted p68/Drosha interaction. Taken together, these data support the idea that mutp53 might exert its GOF activity being responsible for the miRNA downregulation present in cancer.

In order to identify new mechanisms underlying mutp53 GOF activity associated with dysregulation of microRNA in cancer, we have performed a genome wide analysis of miRNA expression in colorectal adenocarcinoma SW480 before and after mutp53 depletion. Our preliminary results revealed that mutp53 depletion is associated with up-regulation of 31 mature miRNAs (corresponding to 41 miRNA genes) and down-regulation of only 3 miRNAs.

Validation of genome wide miRNA expression profile by qRT-PCR analysis for mature forms and primary precursors (pri-miRNAs) shows that mutp53 plays a role both at transcriptional and posttranscriptional level.

All together these preliminary results suggest a main role for mutp53 in the down-regulation of miRNA expression in cancer cells.

P24

Mesenchymal stem cells isolated from different genders and body regions show differences in plasticity linked to the regulation of hox genes

D. Khan¹, Y. Zhang¹, S. Barman¹, A. Pansky¹, B. Brand-Saberi², E. Tobiasch¹

¹Dept of Natural Sciences, Bonn-Rhine-Sieg Univ. of Applied Sciences, Rheinbach, Germany

²Dept of Anatomy and Molecular Embryology, Ruhr-Univ. Bochum, Bochum, Germany

Degenerative diseases are the major cause of death and morbidity in the industrialized world. In these diseases such as in osteoporosis deterioration of tissue occurs. Stem cells are considered to be a promising tool for future repair approaches of the damaged tissue.

Adipose tissue derived stem cells (ATSCs) have been isolated from various regions of the body. According to the literature they are mesenchymal stem cells (MSCs) and should have generally the potential to differentiate into cell types of different tissues such as osteoblasts, adipocytes and chondrocytes. Here we show that these stem cells isolated from different body regions and genders execute differences in plasticity. MSCs obtained from thigh showed a stronger differentiation towards osteogenic lineage if compared to the cells from belly.

Hox genes are involved in the patterning of the anterior to posterior axis and in segmental identity. These genes play a key role in differentiation and organogenesis during embryonic development and are also expressed in adults. Therefore we investigated if these genes are linked to the discriminative differentiation potential of stem cells isolated from different genders and body regions. We found that HoxA3, HoxB6, HoxB8 and HoxC5 were up-regulated strongly in female donors during osteogenic induction. HoxB6 and HoxC5 were up-regulated during adipogenic differentiation in all tested donors.

We conclude that Hox genes might be good candidate to trigger stem cell differentiation, especially towards the osteogenic lineage.

Correspondence: edda.tobiasch@h-brs.de

P25

Constitutive inhibition of muscle differentiation by senescence-activated DNA damage signaling

L. Latella^{1,3}, M. Simonatto¹, F. Marullo¹, PL. Puri^{1,2}

¹Istituto Dulbecco Telethon, IRCCS Santa Lucia Foundation and European Brain Research Institute, Rome, Italy

²The Burnham Institute for Medical Research, San Diego, USA

³Institute of Translational Pharmacology, National Research Council of Italy, Rome, Italy

Upon exposure to genotoxic stress, skeletal muscle progenitors coordinate DNA repair and the activation of the differentiation program, by the DNA damage-activated “differentiation checkpoint” (DC), which prevents the transcription of differentiation genes during the DNA repair. We have shown that in myoblasts, DNA damage-activated cAbl phosphorylates MyoD at tyrosine 30 (Y30) that transiently inhibits MyoD-dependent transcription following DNA damage, and is reversed possibly upon the successful repair of the lesion (1). The presence of a cAbl consensus site discriminates MyoD from the functional paralog Myf5 and from other muscle bHLH proteins in executing the DC (2).

In this study we show that the constitutive, endogenous DNA damage signaling associated with cellular senescence triggers a persistent DC that constitutively inhibits the myogenic program induced by MyoD. Indeed, replicative senescence fibroblasts become progressively resistant to MyoD-mediated myogenic conversion, in coincidence with the senescence-activated DNA damage signaling. Conversely, abrogating the DC response by using the Y30F-MyoD unresponsive to cAbl phosphorylation, we show that senescent cells are again proficient to differentiate.

We also observed that the ability of Y30F-MyoD to bypass the constitutive DC elicited in senescent cells and to convert senescent fibroblasts is coupled with the induction of the cell cycle from a G0 senescent state. The transition into a new round of DNA replication may serve to make competent tissue-specific chromatin loci for gene expression.

These results indicate a close coordination between DNA damage-activated checkpoints, progression into the cell cycle and control of tissue-specific gene expression, and reveal a novel role of the DNA damage signaling as a key mediator of the functional antagonism between cellular senescence and terminal differentiation. Moreover our data underscore an emerging new role of MyoD in regulating DNA synthesis as a critical phase to epigenetically determine cellular fates.

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P26

Cytotoxic effects induced by JA47, a novel histone deacetylase inhibitor (HDACi), on MDA-MB231 breast cancer cellsM. Librizzi¹, A. Longo¹, M. Agnello¹, J. Amin², J. Spencer³, C. Luparello¹¹Dipartimento STEM BIO, Univ. di Palermo, Italy²School of Science at Medway, Univ. of Greenwich, United Kingdom³Dept of Chemistry, Univ. of Sussex, United Kingdom

It is acknowledged that epigenetic alterations are involved in the repression of tumor suppressor genes and the promotion of tumorigenesis in cancers; for this reason, novel compounds endowed with HDAC inhibitory activity are considered as an attractive anti-cancer therapeutic approach. Here, we describe the biological effects induced by the novel HDACi JA47, an organo-metallic SAHA analogue [1] on viability/proliferation, cell cycle progression, apoptosis/autophagy induction and mitochondrial activity/ROS accumulation of a triple-negative highly-tumorigenic breast cancer cell line, MDA-MB231, taken as an in vitro model system of “aggressive” breast carcinoma. Viability and growth rate were determined using an MTT assay, and the results obtained strongly suggest that JA47 exerted a cytotoxic effect with an IC₅₀ = 8.45 μM at 72 h. Different sets of experiments performed at 24 and 48 h of exposure to JA47 indicate that it induced a non-apoptotic cell death, as evaluated by an annexin assay, characterized by the accumulation of cells in the G1 and subG0 phases of the cycle. Moreover, we observed a prominent reduction of acidic vesicular organelles (AVO), hallmarks of autophagy, in JA47-treated cells, which may be related to a deprivation of energy supply indispensable for tumor cell survival. In addition, we also demonstrated that JA47 affected mitochondrial activity, as shown by a JC-1 assay, and triggered generation of reactive oxygen species; noteworthy, cytotoxicity was reversed in a dose-dependent manner by co-incubation with the anti-oxidant butylated hydroxytoluene. We conclude that JA47 plays a potential anti-tumoral role towards triple-negative breast carcinoma cells via autophagy down-regulation and oxidative injury.

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P27**The expression of PTHrP isoforms in differentiating human fat-derived mesenchymal stem cells**

A. Longo¹, I. Catanzaro¹, F. Caradonna¹, E. Tobiasch², C. Luparello¹

¹Dept STEM BIO (Biologia Cellulare), Univ. of Palermo, Palermo, Italy

²Dept of Natural Sciences Bonn-Rhine-Sieg, Univ. of Applied Sciences, Rheinbach, Germany

Mesenchymal stem cells (MSCs) from fat tissue can differentiate *in vitro* towards osteoblasts and adipocytes [1], and to search for stemness/early differentiation markers, we examined the expression of the splicing isoforms of Parathyroid Hormone-related Peptide (PTHrP), a regulator of proliferation, differentiation and apoptosis. The PTHrP gene, coding for three protein variants of 139, 141 and 173 aa, contains two TATA promoters, P1 and P3, and a GC-rich promoter, P2, and nine exons undergoing to alternative splicing [2]. In MSCs we found four mRNAs encoding for the 139 and 173 aa isoforms, whereas osteo-differentiating cells produced only two mRNAs encoding for the same protein isoforms, and adipo-differentiating cells only one mRNA encoding for the 173 aa isoform. Moreover, P2 was always silenced whereas P3 only in differentiated cells. Our results suggest that during differentiation, the expression of PTHrP isoforms becomes increasingly selective. We also examined the methylation state of P2 and P3 in undifferentiated and osteo-differentiating MSCs, to check the possible correlation between methylation and promoter silencing. In agreement with gene expression data, three CpG island internal sites of P2 were hyper- and partially-methylated in most DNA preparations, and CpG sites of P3 were methylated in differentiated cells. We conclude that PTHrP plays a role in the differentiation of MSCs through the selective regulation of isoforms *via* promoter methylation and that PTHrP isoform expression could be considered a putative marker of MSC differentiation.

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P28

Dissecting the role of Polycomb in protecting the identity of differentiated skeletal muscle cells upon environmental cues

B. Bodega¹, F. Marasca¹, C. Lanzaolo², V. Orlando¹

¹Dulbecco Telethon Institute (DTI), IRCSS Fondazione Santa Lucia, Epigenetics and Genome reprogramming, Rome, Italy

²CNR Institute of Neurobiology and Molecular Medicine, IRCCS Santa Lucia Foundation, Rome, Italy

Polycomb proteins are epigenetic regulators that prevent changes in transcription and maintain the memory of silent genes through cell division. Despite decades of research on Polycomb function, little is still known about its role in terminally differentiated tissues. Skeletal muscle cells (C2C12) represent a paradigmatic differentiation model in which Polycomb complex 2 (PRC2) has been extensively studied. We already found specific dynamics of PRC2 proteins in myoblasts and myotubes, where Ezh1 progressively substitutes the H3K27 methyltransferase Ezh2; moreover, Ezh1 is required for myogenic differentiation and the two complexes (i.e. PRC2-Ezh2 and PRC2-Ezh1) differentially regulate muscle specific genes (early versus late differentiation genes) (Caretto 2004; Stojic 2011). It is recently reported that Ezh1 associates with active gene regulatory regions and promotes RNA pol II elongation in differentiating C2C12; Ezh1 retains a weak H3K27 methyltransferase (HMT) activity, although highly homolog to Ezh2, and its primary function remains elusive (Mousavi 2012). Since H3K27 tri-methylation levels are steady in myotubes and PRC2-Ezh1 complex is correctly formed (Stojic 2012), we asked which could be PRC2 function in this context. Our working hypothesis stems from the observation that upon oxidative stress (i.e. H₂O₂ treatment), global level of H3K27me₃ specifically increased; ChIP data indicated increased levels of H3K27me₃ on the regulatory regions of Ezh1 target genes, together with their reduced expression upon H₂O₂ treatment. Moreover, we detected by chromatin fractionation an Ezh1 enrichment on chromatin fraction, suggesting its functional re-localization. RNA-seq and ChIP-seq (Ezh1, Suz12, H3K27 me₃) analyses are ongoing in H₂O₂-treated myotubes. Hence we are addressing the possibility that PRC2-Ezh1 complex could exhibit its repressive function in myotubes as a signal-dependent response to protect post-mitotic cells identity from environmental cues.

P29

A genome wide role for nuclear Dicer1 and Ago1 in transcriptional regulation in human cells

K.M. Parsi^{*1}, K. Hideya^{*2}, N. Hornig¹, A.M. Burroughs², A. Saxena², C. Daub², Y. Hayashizaki², P. Carninci², V. Orlando¹

¹Dulbecco Telethon Institute, Epigenetics and Genome Reprogramming, Rome, Italy

²RIKEN Omics Science Center, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa, Japan

*These authors contributed equally to this work

Small RNAs and RNA interference (RNAi) components have emerged as key effectors of pathways that control gene expression. The role of RNAi in the cytoplasm is well documented, but the role of small RNA and RNAi components in the nucleus, especially in animals, remains largely obscure. Here we sought to study genome wide function of RNAi factors Dicer1 and Ago1 proteins in human HepG2 cells. By chromatin immunoprecipitation coupled to high throughput sequencing (ChIP-seq) and RNA sequencing (RIP-seq) we found that human RNAi components Dicer1 and Ago1 strongly associate with promoters and enhancers of active gene loci. Furthermore, by co-immunoprecipitation experiments we found that Dicer1 interacts in vitro with RNA Polymerase II (Pol II) and TFIID in an RNA dependent manner, and also it interacts with enhancer binding proteins, p300 and HDAC2 factors. Finally, loss of function experiments show that Dicer1 and Ago1 function affect the in vivo Pol II chromatin binding and transcriptional output. Our findings suggest that the chromatin associated RNAi components Dicer1 and Ago1 take part in enhancer driven transcriptional activation.

P30

L-Proline controls mouse ESC metastability modulating the epigenetic signature

S. Comes, M. Gagliardi, N. Laprano, A. Cimmino, E.J. Patriarca, M.R. Matarazzo, G. Minchiotti
Institute of Genetics and Biophysics "A. Buzzati-Traverso", CNR, Naples, Italy

Embryonic stem cell (ESC) metastability, i.e. their phenotypic, functional, and molecular heterogeneity, mainly arises from stem cells' ability to dynamically interconvert between different pluripotent states in response to extracellular signals. At the extreme of this plastic phenotypic variation, cells display identifiable features of the two types of mouse embryo-derived pluripotent stem cells, namely ESCs and EpiSCs (Epiblast Stem Cells), deriving from the inner cell mass and late epiblast or primitive ectoderm, respectively. To date, factors and molecular mechanisms controlling mESC metastability are far to be fully resolved.

We have recently reported that L-Proline forces ESCs toward a novel EpiSC-like metastable state, namely L-Pro-induced cells (PiCs). Remarkably, unlike EpiSCs, PiCs rely on leukemia inhibitor factor to self-renew, colonize the embryo following blastocyst injection, and rapidly revert to an ESC state after L-Pro removal.

Global gene expression analysis revealed dramatic changes in the transcriptional profiling of PiCs compared to ESCs. Interestingly, several histone modifier enzymes resulted deregulated, suggesting that L-Proline triggers epigenetic changes. In line with this hypothesis, our previous data showed that the ascorbic acid (VitaminC), a cofactor of the Jumonji-histone demethylases, antagonizes ESCs↔PiCs phenotypic transition, in a dose dependent manner. Most remarkably, PiCs show several phenotypic similarities with Jmjd1a and Jmjd2c knockdown ESCs, such as flat morphology, sensitivity to trypsin digestion as well as upregulation of specific genes.

All together, our findings prompted us to investigate whether L-Pro treatment can induce changes in the epigenetic signature of ESCs. Here we report that L-Pro affects the level of both global and gene-specific histone lysine methylation state of ESCs, and propose a novel mechanism controlling ESC metastability, through L-Pro –dependent modulation of the epigenetic signature.

P31

Transcriptional regulation of the oncosuppressor miR-342 and let-7c in myeloid cells

S. Careccia¹, A. Pelosi¹, M. Levrero², G. Piaggio¹, MG. Rizzo¹

¹Dept of Experimental Oncology, Laboratory of Molecular Oncogenesis, Regina Elena Cancer Institute, Rome, Italy

²Dept of Internal Medicine, Gene expression Laboratory, Univ. "Sapienza" of Rome, Italy

MiR-342 and let-7c are intronic microRNAs (miRNA) that can act as tumor-suppressor genes targeting oncogenes. Our previous studies indicate that in acute promyelocytic leukaemia (APL), a peculiar subtype of acute myeloid leukaemia (AML) bearing the leukaemia promoting PML/RAR α fusion protein, miR-342 and let-7c are down-regulated in APL blasts at diagnosis, compared to in vitro-differentiated normal promyelocytes and their expression increase after ATRA treatment. However, the molecular mechanisms responsible for the modulation of their expression is still not characterized. Here we focus on the transcriptional regulation of these two miRs. We found that DNA methylation of host gene promoters does not appear to be involved in the regulation of these miRNAs while posttranslational status of histones have been observed upon ATRA treatment. Notably, we have identified for let-7c a new transcriptional start site (TSS) in an intron of host-gene and up-stream of the pre-miRNA. The new promoter region contains canonical TATA and CCAAT box elements. Interestingly, we have identified for let-7c a new transcriptional start site in addition to host-gene promoter. This new promoter has an own transcriptional activity and ATRA treatment induces, as for the host-gene promoter, an open chromatin configuration with an enrichment of p300 and an increase of epigenetic marks that correlate with a more active transcriptional state. Interestingly, we also show that the intragenic promoter drives transcription of let-7c depending upon the tumor hystotype. Of note, we also provide evidence that the intragenic promoter drives transcription of let-7c depending upon the tumor hystotype. These results support the model that ATRA-induced up-regulation of miR-342 and let-7c in APL cells may be mediated by epigenetic events.

P32

Identification of A-to-I RNA editing sites in human brain and spinal cord by RNA-Seq technology

E. Picardi^{1,2}, A. Gallo³, S. Raho³, F. Galeano³, S. Tomaselli³, G. Pesole^{1,2}

¹Univ. di Bari "A. Moro", Dipartimento di Bioscienze, Biotecnologie e Scienze Farmacologiche, Bari, Italy

²Istituto Biomembrane e Bioenergetica del Consiglio Nazionale delle Ricerche, Bari, Italy

³Laboratorio di ricerca RNA editing, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy

RNA editing is a widespread epigenetic mechanism in which primary transcripts are modified at specific positions. It occurs in a variety of organisms including human and cooperates with alternative splicing in increasing both proteomic and transcriptomic complexity. RNA Editing can modulate gene expression and lead to altered protein functionality. Indeed, its deregulation in human has been linked to several neurological diseases. To date many editing events have been identified by next generation sequencing technologies employing massive transcriptome and genome sequencing. While this is the optimal way to detect edited positions, genome and transcriptome reads from single human individuals are not always available. In contrast, numerous RNA-Seq experiments are stored in public databases and represent a relevant source of yet unexplored RNA editing sites.

To identify de novo RNA editing sites in RNA-Seq data we developed an easy computational strategy based on a double mapping procedure in which millions of short reads were independently mapped onto the human transcriptome and the reference human genome, retaining only concordant alignments for downstream analyses. Mapped reads were explored to calculate the empirical probability to observe a substitution. Such probabilities were then used to detect statistically significant base conversions by applying the Fisher's exact test by comparing the observed and expected occurrences in the aligned reads.

Applying our double mapping procedure on the SRA study SRA012427 from human brain we found 19 highly significant A-to-I conversions in known human coding regions. Interestingly, 11 of such changes have been already described in literature and 6 were experimentally confirmed by Sanger sequencing. We further corroborated our strategy in human spinal cord performing a RNA-Seq experiment in which we confirmed 12 out of 15 RNA editing candidates by whole exome sequencing carried out on the same individual and tissue.

P33

Adolescent THC exposure influences chromatin remodeling

P. Prini, E. Zamberletti, S. Speziali, M. Gabaglio, T. Rubino, D. Parolaro

Dept DISTA, Univ. of Insubria, Busto Arsizio (VA)

We recently demonstrated that adolescent THC treatment triggers the development of a complex depressive-like phenotype in adult female rats. Since recent evidence suggests that epigenetic mechanisms may underlie the development of mental illnesses, in the present study we checked whether epigenetic alterations could underlie the THC-induced depressive-like phenotype.

To this aim, female rats were treated with increasing doses of THC during adolescence (PND 35-45). Twenty-four hours after the last THC injection, acetylation of Lysin14 and trimethylation Lysin27 levels, both on histone H3, were investigated in the PFC, NAc, Hippo and Amy, some of the brain areas more involved in the modulation of emotional behavior.

To clarify whether adolescence really represents a more vulnerable period for the long-term adverse effect of THC, the same treatment protocol and subsequent biochemical analyses were performed at adulthood (PND 75-85).

Adolescent THC exposure significantly increased H3K14Ac levels in all the areas considered, without altering H3K27-3met levels. In contrast, adult THC exposure only altered H3K14Ac in NAc, whereas a significant decrease in H3K27-3met levels was present in the PFC.

These results suggest that THC exposure triggers different epigenetic alterations depending on the time window of administration. Interestingly, adolescent exposure promotes gene transcription through an increase of H3K14Ac levels.

In order to provide information about the genes whose transcription was altered by adolescent THC exposure, we measured mRNA levels of the cannabinoid CB1 receptor and some genes involved in brain development (i.e. PSD95, BDNF and CREB).

In the PFC, both CB1 and PSD95 mRNA were significantly enhanced, whereas no changes were found in BDNF and CREB mRNA.

These data indicate that THC treatment triggers different epigenetic modifications in adolescent and adult animals. These preliminary data suggest that adolescent THC-induced chromatin alterations impact mRNA expression of genes involved in brain development.

P34

Genome-wide analysis of chromatin Poly-ADP-Ribosylation in *Drosophila melanogaster*

A. Sala^{1,2}, D.F.V. Corona^{1,2}

¹Dulbecco Telethon Institute c/o ²Univ. degli Studi di Palermo, Dipartimento STEM BIO, Sezione Biologia Cellulare, Palermo, Italy

Poly-ADP-Ribosylation is a post-translational modification of proteins mediated by Poly-ADP-Ribose Polymerases (PARPs). Using NAD⁺ as a substrate, PARPs catalyze the covalent attachment of ADP-ribose units on target proteins, to generate long linear and branched Poly-ADP-Ribose (PAR) chains. PARPs are involved in the regulation of critical cellular functions, including transcriptional regulation.

While it is widely demonstrated the accumulation of PAR at decondensed and transcriptionally active loci of highly inducible genes, very little is known about basal PARylation function in transcription in the absence of induced stimuli.

Unlike mammals, which have several PARP encoding genes, the model organism *D.melanogaster* has only one PARP gene, highly related to mammalian PARP-1, making flies a great model system to study PARP biology. In order to study the role of PARylation on chromatin in non-induced conditions we conducted a genome-wide analysis of PAR distribution on *Drosophila* chromosomes.

Our analysis revealed that PAR has ~5600 high affinity chromatin binding sites, both in genic and intergenic regions. In order to check if PAR binds a particular group of genes involved in specific biological processes, we conducted an analysis of PAR-bound genes based on their Gene Ontology (GO) classification. This GO analysis revealed that PAR binds genes encoding for factors involved in a variety of essential biological functions and that PAR-bound genes were over represented in genes encoding factors involved in "Signal Transduction".

Moreover, in order to identify genes whose expression is altered when the in vivo amount of PAR is modified and to correlate ChIP-chip binding data with expression data I carried out *Drosophila* whole-genome expression microarray analysis.

P35

Differential effects of Ha-RasG12V, Ki-RasG12V and Ki-RasG13D on cell proliferation

M.R. Saladino, F. Miranda, I. Albanese

Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO), Univ. di Palermo

Although differing only for the last 24 aminoacids, the three major isoforms of p21 Ras (Ha-, Ki- and N-Ras) can trigger alternative pathways of signal transduction, at least in part as a consequence of different post-translational modifications and subcellular localization. Ras mutations are a common event in tumorigenesis. In colorectal carcinomas (CRCs) the mutations affect almost exclusively Ki-Ras, while Ha-Ras mutations are mostly found in bladder carcinomas and N-Ras mutations in leukemia cells. In almost all cases, the genetic alteration is a point mutation in codons 12 or 13, and less frequently in codon 61. By affecting the GTPase activity of the protein, they always lead to a constitutively active protein. However, data obtained in different experimental systems or by analysis of primary and metastatic tumors show that mutations in different codons, different mutations in the same codon, and mutations of different isoforms of Ras may have diverse biological consequences. To shed more light on the molecular mechanisms responsible for the different effects of Ras mutations, we have obtained stable clones of HT-29 (a human colorectal adenocarcinoma cell line in which the endogenous Ras genes are wild type) transfected with cDNAs codifying Ha-RasG12V, Ki-RasG12V and Ki-RasG13D, under the control of an hormone-inducible promoter. The expression of each of these mutated Ras isoforms induces specific, different effects on cell morphology and growth rate. FACS analysis shows also a differential effect on the cell cycle. H-RasG12V expression, in addition, seems to determine apoptosis. Despite these differences, all three mutated isoforms of Ras increase the expression of the CDK inhibitor p21. Preliminary data reveal epigenetic changes in the p21 promoter region upon induction of H-RasG12V expression.

P36

Replication-independent expression of H1° and H3.3 histone variants is probably regulated by different RNA-binding proteins

P. Saladino¹, C.M. Di Liegro¹, P. Proia², G. Schiera¹, I. Di Liegro³

Depts of ¹Scienze e Tecnologie Molecolari e Biomolecolari, ²Studi Giuridici, Economici, Biomedici, Psicosociopedagogici delle Scienze Motorie e Sportive, and ³Biomedicina Sperimentale e Neuroscienze Cliniche, Univ. of Palermo, Palermo, Italy

DNA in eukaryotes is wrapped around core histones to form nucleosomes, the basic units of chromatin. The linker histones H1 bind DNA where it enters and leaves the nucleosome, thus stabilizing higher order structures. Chromatin is a dynamic complex, modulated by different processes such as DNA-methylation, post-translational modifications of histones, and incorporation of specific histone variants. Throughout rat brain development, expression of H1° and H3.3 histone variants is mainly regulated at the post-transcriptional level. These proteins are of interest for their possible involvement in the replication-independent chromatin remodelling induced by extracellular stimuli.

We previously cloned two cDNAs encoding, respectively, PIPPin (or CSD-C2), a brain-enriched protein able to bind the 3' end of both H1° and H3.3 mRNAs, and LPI (longer isoform of PEP-19). Both PEP-19 and LPI are brain-specific. By western blot, we found that PIPPin expression in PC12 cells is enhanced by NGF-induced differentiation. We investigated the RNA-binding properties of the three proteins using their 6 histidine-tagged recombinant fusions and found that they all bind H1° and H3.3 RNAs. Since PEP-19 and LPI are camstatins, we also analyzed whether calmodulin could interfere with RNA-binding, and found that calmodulin competes with H1° RNA binding to both proteins, while it is not able to bind RNA on its own. This finding suggests that, in the brain, PEP19 and LPI could induce histone mRNA translation in response to calcium.

By using biotinylated H1°/H3.3 RNA as fishing molecules, we isolated by affinity chromatography a group of proteins which were analyzed by mass spectrometry. Among them some heterogeneous nuclear ribonucleoproteins (HnRNP K, A1, A2/B1) and the Hsc70 chaperone. We are currently studying the interactions among these proteins by co-immunoprecipitation assays.

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P37

Role of microbiota in development, differentiation and epigenetic modifications of model organism *D. melanogaster*

R. Sanfilippo^{1,2}, S. Campora², A. M. Puglia², R. Alduina², A. Cuomo³, T. Bonaldi³, T. Vaccari⁴,
D. Corona^{1,2}

¹Dulbecco Telethon Institute c/o ²STEMBIO Dept, Univ. of Palermo, Italy

³Dept of Molecular Oncology, European Institute of Oncology, Milano, Italy

⁴IFOM – FIRC Institute of Molecular Oncology, Milano, Italy

The transcriptional status of a gene is tightly linked to the structure of its chromatin. The N-termini of the four core histones protrude out of nucleosome, making them available for a variety of covalent post-translational modifications: specific docking sites for proteins regulating chromatin structure and gene transcription that account for the “histone-code”.

Despite data concerning the ability of some pathogens to manipulate host gene expression by modulating chromatin accessibility, relatively little is known about the role of the resident Microbiota.

Using both conventionally reared as well as germ-free *D. melanogaster* as a model, it was demonstrated the involvement of such bacteria in host protein expression and development. The aim of this PhD project is to clarify the molecular mechanisms underlying these differences. SILAC metabolic labeling and mass spectrometry analysis will be used to recognize differentially expressed proteins.

Using both genetic and biochemical approaches, differentially expressed genes coding for those proteins will be identified and their epigenetic modifications will be studied. Furthermore the bacterial strain able to modulate host gene expression will be detected through germ-free flies colonization experiments. These findings will provide crucial insights into the correlation between *Drosophila* microbiome activity and host metabolic homeostasis.

P38

A novel Oct4-Rb1 axis is orchestrated by miR-335 to control mESC self-renewal and differentiation

S. Schoeftner^{1,2,†}, M. Scarola^{1,2}, E. Comisso^{1,2}, C. Schneider^{1,3}, R. Benetti^{1,2,3}

¹Laboratorio Nazionale Consorzio Interuniversitario Biotecnologie (LNCIB); Area Science Park; Padriciano 99, Trieste, Italy

²Laboratorio Nazionale Consorzio Interuniversitario Biotecnologie (LNCIB), Oncoepigenetic Unit, Area Science Park, Padriciano, Trieste, Italy

³Dipartimento di Scienze Mediche e Biologiche, Università degli Studi di Udine, Udine, Italy

†current address: Italian National Cancer Institute, Regina Elena, Rome, Italy

The pluripotency of mouse embryonic stem cells (mESC) is controlled by a complex network of transcription factors, miRNAs and signaling pathways. Here, we provide evidence for a new regulatory circuit that connects miR-335, Oct4 and the Retinoblastoma pathway to control mESC self-renewal and differentiation. We found that Oct4 drives the expression of Nipp1 and Ccnf that inhibit the activity of the protein phosphatase 1 (PP1) complex to ensure hyper-phosphorylation of the Retinoblastoma protein 1 (pRb) as a key feature of rapidly proliferating, self-renewing mESCs. This introduces the Oct4/Nipp1/Ccnf/PP1/Rb1 axis as a novel pathway that promotes mESC self-renewal. Importantly, miR-335 can interfere with this regulatory circuit by targeting conserved sequence motifs in the 3'UTR of Oct4 and Rb1. The robust upregulation of miR-335 upon induction of mESC differentiation co-operates with the transcriptional repression of Oct4 to facilitate the collapse of the Oct4/Nipp1/Ccnf/PP1 axis, resulting in pRb de-phosphorylation, the exit from self-renewal and the establishment of a pRb regulated cell cycle program of differentiated cells. In line with this model, ectopically increased miR-335 levels impair self-renewal and accelerate mESC differentiation - preferentially towards mesoderm. Our results introduce Oct4 dependent control of the Rb pathway as novel regulatory circuit regulating mESC self-renewal and differentiation.

P39**Mechanisms of altered transcriptional regulation in the pathogenesis of AEC syndrome**G. Ferone^{1,2}, A. Sirico¹, C. Missero^{1,2}¹CEINGE Biotecnologie Avanzate, Napoli, Italy²Fondazione IRCCS SDN, Napoli, Italy

The p53 homologue p63 is a tetrameric transcription factor with a pivotal role in stratified epithelial development and maintenance. Heterozygous mutations in p63 cause at least five closely-related inherited disorders, among which Ankyloblepharon-Ectodermal dysplasia-Clefting (AEC) syndrome is characterized by severe involvement of the skin. To elucidate the molecular mechanisms underlying this disorder, we previously generated a knock-in mouse model (p63^{+/L514F}) carrying a clinically relevant missense mutation in the carboxyl-terminal region of the p63 α isoform, in which AEC mutations are clustered. p63^{+/L514F} mice are affected by cleft palate, skin hypoplasia and ectodermal dysplasia, thus faithfully recapitulating the phenotype observed in AEC syndrome. Cleft palate and skin hypoplasia are caused by hypoproliferation of epithelial progenitors and reduced number of epidermal stem cells associated with downregulation of FGF signalling and FGFR2 expression.

Mechanistically we find that mutant p63L514F protein retains the ability to form tetramers with itself and the wild-type protein, however the tetramer has an impaired transactivation activity. In mutant cells reduced transactivation is associated with partially impaired p63 binding to DNA in the chromatin context and a modest impairment of tri- and di-methylation of histone H3 at lysine 4 at the p63 binding sites. Consistently, in the absence of the wild-type protein, p63L514F is severely impaired in its ability to bind DNA in the genomic context and is unable to transactivate its target genes. These findings indicate that mutant p63 acts in a dominant-negative fashion affecting p63 ability to transactivate crucial targets in epidermal development.

P40

Role of the chromatin remodeling factor ISWI in the *Drosophila* germline stem cells identity

M. Toto^{1,2}, D.F.V. Corona^{1,2}

¹Dulbecco Telethon Institute c/o ² Univ. degli Studi di Palermo, Dipartimento STEMBIO, Sezione Biologia Cellulare, Palermo, Italy

The balance between stem cell self-renewal and differentiation is precisely controlled to ensure tissue homeostasis as well as to prevent tumorigenesis. Regulation of stemness, which depends on both tissue-specific transcriptional regulation and chromatin organization modulation, is still poorly understood. It has been shown that in *Drosophila* ovary, the evolutionary conserved chromatin remodeling factor ISWI maintains germ stem cell (GSC) identity repressing the expression of the differentiation-promoting bag of marbles (*bam*) gene. Interestingly, we showed that loss of ISWI function in *bam* mutant female GSCs suppresses the hyperproliferation of GSCs typically observed in a *bam* mutant ovary, suggesting a role of ISWI in maintaining GSC self-renewal in *bam*-independent way. To dissect ISWI role in controlling stem cell self-renewal we screened for mutations in genes that modify ISWI GSC defects. Our study identified two evolutionary conserved genes *tramtrack* and *effete* capable of suppressing the defects observed in ISWI mutant GSCs. These results underline that the mechanism by which ISWI controls GSC self-renewal and proliferation do not depend exclusively on *bam* but can be influenced by a variety of nuclear factors that modulate ISWI chromatin remodeling activity.

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HIV-Tat interaction with NFκB signaling: upregulation of pro-inflammatory genes expression

E. Vecchio, G. Fiume, A. de Laurentiis, F. Trimboli, C. Palmieri, C. Falcone, F. Fasanella Masci, M. Pontoriero, G. Scala, I. Quinto

Dept of Experimental and Clinical Medicine, Univ. of Catanzaro "Magna Graecia", Germaneto-Catanzaro, Italy

Background: Enhanced NFκB activity is present in HIV-1-infected cells leading to up-regulation of NFκB-dependent expression of pro-inflammatory genes. We investigated whether the HIV-1 Tat transactivator sustains the NFκB activity by deregulating the post-activation turn-off of NFκB, which is mainly dependent on the de novo synthesis of the IκBα repressor.

Results: Tat inhibited the post-activation turn-off of NFκB in single round infection of Jurkat cells with HXB2-env-pseudotyped HIV-1 virions, as well as in PMA-stimulated HeLa cells transfected with Tat. In PMA-stimulated HeLa cells, Tat bound to IκBα and promoted the release of p65 from the complex with IκBα. Moreover, Tat counteracted the IκBα inhibition of p65 DNA binding and transcriptional activities as shown by EMSA and transient expression assays with the κB-Luc reporter. The cysteine-rich domain of Tat and the Rel Homology Domain of p65 (1-121 aa) were involved in physical interaction of the two proteins. A further analysis of Tat action on multiple gene expression revealed a strong induction of MIP1α expression in Tat-transfected HeLa cells, which was mediated by p65. ChIP analysis showed the recruitment of p65 at the level of selected NFκB enhancers in Tat-transfected cells, and the Tat occupancy of selected NFκB-dependent promoters concomitantly with the IκBα removal from the same sites. **Conclusions:** Based on this study, Tat hijacks the IκBα inhibitor and associates with p65 to increase and sustain the NFκB activity. The cross talk of Tat with the NFκB pathway here described suggests novel therapeutic strategies for counteracting the Tat and NFκB-dependent abnormal inflammatory response in AIDS.

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P2 receptors influence human adipose tissue derived mesenchymal stem cells differentiation towards endothelial and smooth muscle cells

Y. Zhang, D. Khan, M. Lange, A. Pansky, E. Tobiasch

Dept Natural Science, Bonn-Rhine-Sieg Univ. of Applied Science, Rheinbach, Germany

Beside the differentiation potential into adipogenic, osteogenic and chondrogenic cell lineages, human adipose tissue derived-mesenchymal stem cells (hATSCs) have also shown the ability to differentiate towards endothelial cells (ECs) or smooth muscle cells (SMCs) recently, which attracted increased attention for the use of hATSCs in vascular system for Regenerative Medicine.

P2 receptors are an old evolutionary but newly characterized family of membrane receptors binding ATP, UTP or their breakdown products as ligands. They play an important role in vascular activities. Our recent publication has shown that P2 receptors influence adipogenic and osteogenic differentiation of hATSCs.

In this study, the influence of purinergic signaling on the differentiation process of hATSCs towards ECs and SMCs were investigated and verified via RT-PCR, Western blot and immunochemical staining with EC and SMC specific markers. P2 receptors not only were presented in undifferentiated and differentiated cells, but also show a strongly regulated expression level for various types of the purinergic receptors. These expression levels seem to have intensively linked to the differentiation process, similar to influence of these receptors during the differentiation process towards the osteogenic and adipogenic lineage.

Here we show for the first time that P2 receptors are a major factor in regulating hATSCs differentiation towards ECs and SMCs, which gives new insight in the mesenchymal stem cell (MSC) differentiation processes and might be utilized for controlling the MSC fate in the future.

Correspondence: edda.tobiasch@h-brs.de

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Role of specialized DNA polymerases in promoting survival of tumor

E. Zucca¹, G. Dianov², G. Maga¹

¹Institute of Molecular Genetics IGM-CNR, Pavia, Italy

²Gray Institute for Radiation Oncology & Biology, Univ. of Oxford, United Kingdom

Specialized DNA polymerases are required for translesion synthesis (TLS) to bypass DNA damage lesions. Their over-expression or increased activity, however, could also result in enhanced TLS capability, allowing cancer cells to better cope with the high environmental stress. The biological significance of deregulation of the DNA repair and TLS pol λ in human cancer cells is an open question, so its expression in response to different stress conditions and at different cell transformation steps was tested. We confirmed that pol λ expression is induced by oxidative damage and DNA strand breaks, but the new emerged feature was that pol λ seems to be required for the normal progression through the S-phase. Transient knockdown with RNAi and an inducible knockdown cell line of pol λ were used to check the effects of pol λ impairment on cell vitality and stress response ability. Flow cytometry analysis of DNA content highlighted an S-phase delay in pol λ deficient cells, confirmed by an increase in Chk1 and γ -H2AX phosphorylation, that may be due to accumulation of DNA damage. Replicative stress due to HU treatment and checkpoint inhibition by UCN-01, showed significant differences between knockdown and pol λ -proficient cells, suggesting a possible replication-coupled repair mechanism driven by pol λ .

Pol λ deficiency, thus, induces the checkpoint activation even without DNA damage. The block of S-phase checkpoint by UCN-01 in the presence of pol λ knockdown and replication stress due to HU promotes a 75% of cell mortality during the next cell cycle.

In our proposed model, accumulated DNA damage due to HU treatment in pol λ -deficient cells cannot be repaired if checkpoint activation is inhibited by UCN-01, leading to cell death.

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