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20-21 DICEMBRE 2010

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Dott.ssa Emanuela Craparo, Prof. Antonino Lauria, Dott. Mariano Licciardi, Prof. Paolo Lo Meo

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Dipartimento di Biologia Cellulare e dello Sviluppo "A. Monroy"

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Programma

Lunedì 20 Dicembre 2010

9:25-9:45 Apertura dei Lavori: Prof. G. Spinelli, con interventi dei Proff. G. Cavallaro, G. Cirrincione, R. Noto

Sessione I - Moderatore: V. Cavalieri

9:45-10:00	M. Zizzo	DBCS	Guanine-based purines affects the enteric cholinergic neurotransmission via a mechanism not involving membrane receptors
10:00-10:15	E. Craparo	DCTF	Nanotechnologies for biomedical applications
10:15-10:30	E. Carra	DBCS	Mitochondrial DNA deletions and male infertility
10:30-10:45	A.M. Almerico	DFTB	The long way from molecular modeling to biological screening: the discovery of new HSP90 inhibitors
10:45-11:00	M. Saverini	DBCS	Genotoxicity of terpenes present in wastwater of a citrus transformation factory in bacterial and mammalian cells and effectiveness of photocatalytic degradation
11:00-11:15	R. Noto	DCO	Studies about the effects that ordered structures have on properties of organic substrates

11:15-11:30 Coffee Break e Poster Session nello spazio antistante l'aula Mutolo

Sessione II - Moderatore: M. Ragusa

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12:00-12:15	G. Fontana	DCO	New methodologies in the synthesis and chemical transformation of organic natural products
12:15-12:30	D. Corona	DBCS	Biochemical and genetic regulation of chromatin remodelling
12:30-12:45	D. Schillaci	DCTF	Bacterial biofilms: a challenge in the discovery of new anti-infective agents
12:45-13:00	G. Ghersi	DBCS	Recombinant proteolytic enzymes synthesis. Cell therapy applications

13:00-14:30 Poster Session nello spazio antistante l'aula Mutolo

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15:30-15:45	C. Di Liegro	DBCS	Histone RNA-binding proteins in nerve cell differentiation
15:45-16:00	V. De Caro	DCTF	Buccal delivery as a new challenge for treatment of motor fluctuations in Parkinson Diseases
16:00-16:15	S. Rosselli	DCO	Studies on secondary metabolites from plants

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Martedì 21 Dicembre 2010

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12:30-12:45	V. Barra	DBCS	Bypass of G1 arrest induced by *DNMT1* post-transcriptional silencing triggers aneuploidy in human cells

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*Dipartimento di Biologia Cellulare e dello Sviluppo, Viale delle Scienze, I-90128 Palermo; ‡Dipartimento di Medicina Sperimentale, Corso Tukory 129, I-90134 Palermo, Italia 1

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Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo Viale delle Scienze, Palermo, Italy; 2

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*Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, **Dipartimento di Studi Giuridici, Economici, Biomedici, Psicosociopedagogici delle Scienze Motorie e Sportive, ***Dipartimento di Biologia Cellulare e dello Sviluppo, University of Palermo, Palermo, Italy 23

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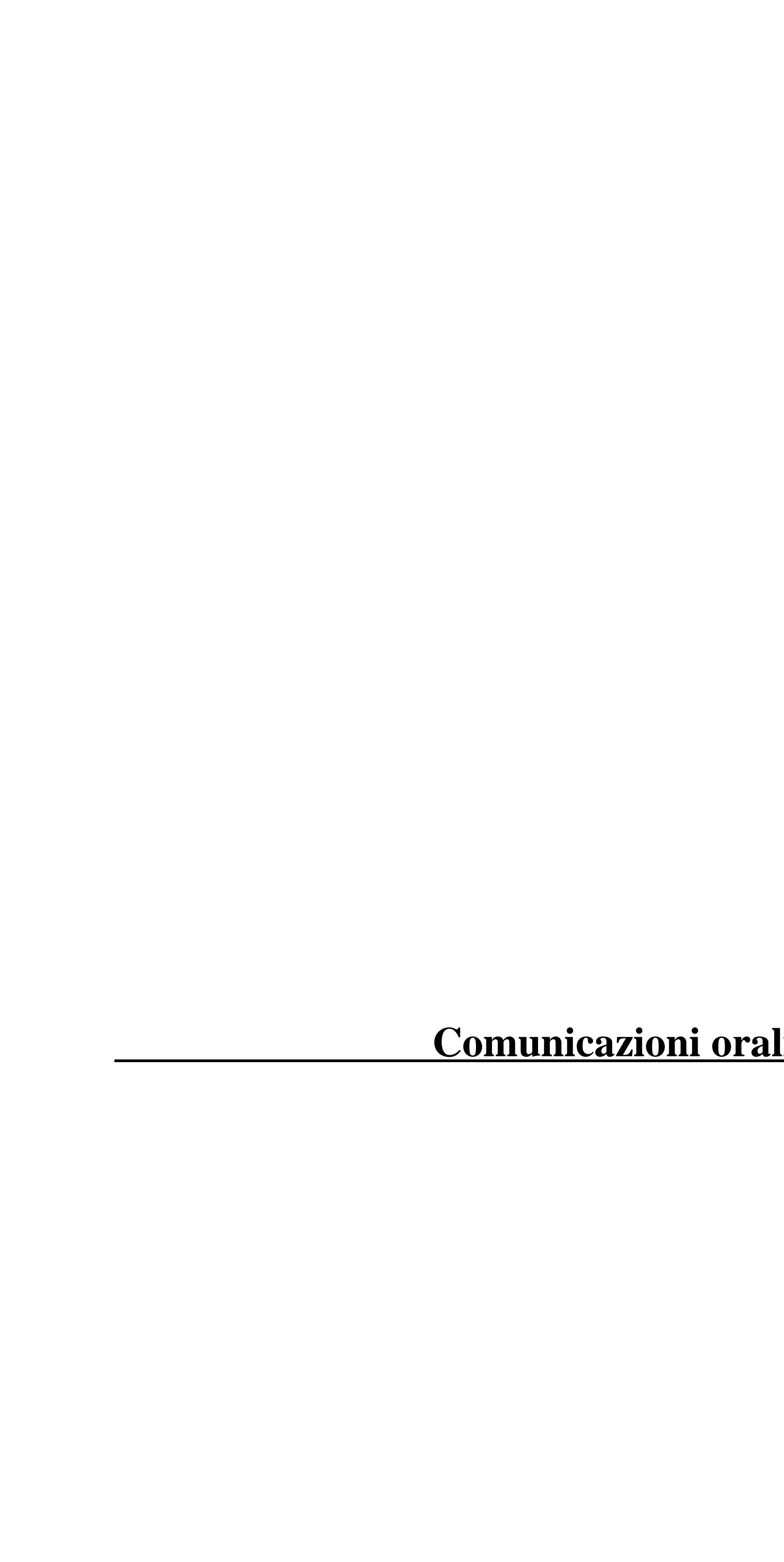
[1]Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo.
[2] Dipartimento di Ingegneria Industriale (D.IN.I) Università di Palermo. [3] LIMA Lto, SpA. Udine 24

Babesia bigemina surface antigens: an overview. *Blanda[1] V., Albanese[1] I., La Farina[1] M., Sireci[3] G., Agnone[3] A., Torina[2] A..* 1 University of Palermo, Dipartimento di Biologia Cellulare e dello Sviluppo
2 National Reference Centre for Anaplasma, Babesia, Rickettsia and Theileria (CRABaRT), Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri". 3 University of Palermo, Dipartimento di Biopatologia e Metodologie Biomediche 24



Comunicazioni orali

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Guanine-based purines affect the enteric cholinergic eurotransmission via a mechanism not involving membrane receptors.

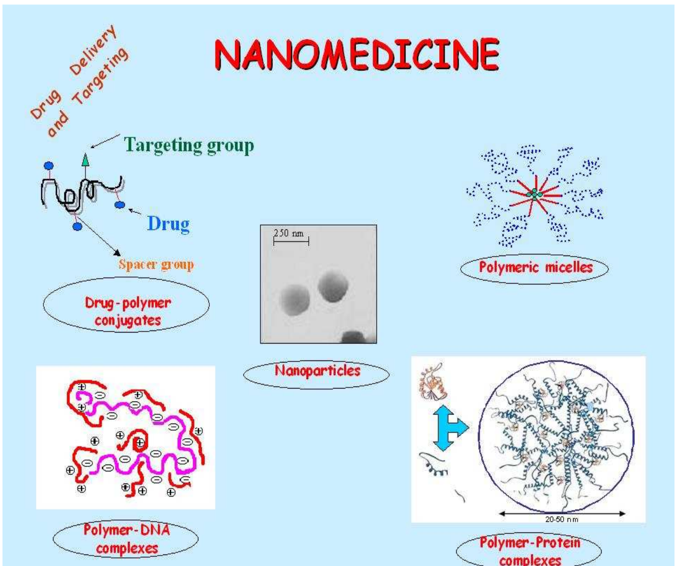
MG Zizzo, F Mule'*, M Mastropaolo*, N Belluardo‡, R Serio**
*Dipartimento di Biologia Cellulare e dello Sviluppo, Viale delle Scienze, I-90128 Palermo;
‡Dipartimento di Medicina Sperimentale, Corso Tukory 129, I-90134 Palermo, Italia

Increasing evidence indicate that guanine-based purines, known as modulators of intracellular processes, can exert extracellular effects, raising the possibility of the existence of specific receptors for these compounds. We investigated if guanine-based purine receptors may be present in the rodent gastrointestinal tract modulating intestinal contractility, as the well known adenine-based purine receptors. Experiments were performed in vitro recording spontaneous and neurally-evoked contractile activity, as changes in isometric tension, in mouse distal colon circular muscle.
Guanosine up to 3 mM or guanine up to 1 mM, did not affect the spontaneous mechanical activity, but they significantly and reversibly reduced the amplitude of the nerve evoked cholinergic contractions. Both compounds did not affect the direct contractile responses to muscarinic agonist. No desensitization of the response was observed. Guanine-based purine effects were not modified by adenine-based purine receptor antagonists or by adenylyl or guanilyl cyclase inhibitors. Dipyridamole or NBTI, nucleoside uptake inhibitors markedly reduced the guanosine effects whilst guanine effects were prevented in the presence of adenine, competitive inhibitor of nucleobase uptake.
Our data indicate that guanosine and guanine are able to modulate negatively the excitatory cholinergic neurotransmission in the mouse colon circular muscle. Guanine-based purines appear to act on prejunctional release of acetylcholine. Their effects are dependent by their cellular uptake, and independent by adenine based purine receptors.

Nanotechnologies For Biomedical Aplications

Personale strutturato:G. Cavallaro, M. Licciardi, E. F. Craparo, G. Giammona
Dottorandi: G. Amato, C. Scialabba, G. Teresi
Laboratorio di Polimeri Biocompatibili, Dpt. of Chimica e Tecnologie Farmaceutiche, Università di Palermo, Italia

Always more frequently, the research in pharmaceutical field moves its attention on the development of nano-structured drug delivery systems (DDS) able to optimize drug delivery, bioavailability and in general pharmaceutical performance of already known drug molecules. Among polymeric materials, polyaminoacids are a very interesting class of polymers suitable for colloidal DDS production because of their protein-like nature and the possibility of preparation by synthesis. Two very interesting synthetic polyaminoacids employed for the preparation of DDS are: α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) and α,β-polyaspartylhydrazide (PAHy) both derivated from a polysuccinimide (PSI). These polymers, initially proposed as plasma expanders, are water-soluble, non-toxic and non antigenic. The Laboratory of Biocompatible Polymers of the Palermo of University, since many years works on the design, preparation and chemical and pharmaceutical characterization of new nanotechnologies for application in nanomedicine, based on the above mentioned polymers for the modified and targeted drug release[1-5].



Nanostructured systems investigated in this laboratory include main nanomedicine systems, such as (see Figure):

- macromolecular prodrugs (drug-polymer conjugates)[1];
- polymeric micelles[2]
- interaction complexes[3] (polymer/protein complex);
- polyplexes[4] (polymer-DNA complexes);
- nanoparticles[5].

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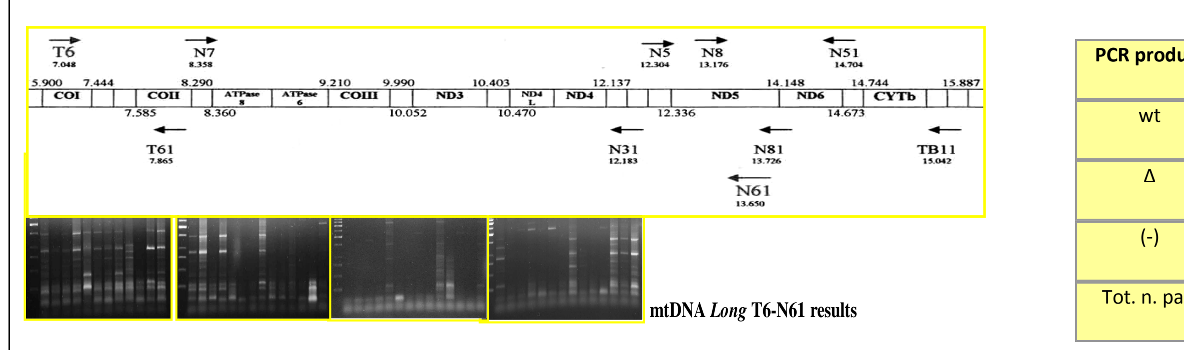
Mitochondrial Dna deletions and male infertility

Salerno Barbara, Gerardi Eliana and Elena Carra
Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo Viale delle Scienze, Palermo, Italy;

In men, oligozoospermia, asthenozoospermia, teratozoospermia and azoospermia are the main causes of infertility. The present research is aimed to investigate if mtDNA deletions can cause sperm defects in idiopathic astenozoospermic patients with different sperm motility and sperm concentration.
The aim of this investigation was to test the hypothesis that whole sperm samples with lower levels of motility would have a higher incidence of spermatozoa with deletions in mitochondrial genome. We were able to evidence ΔmtDNA when the DNA was amplified from the non motile fraction from semen samples or whether DNA from whole sperm samples was employed long PCR.
In conclusion this study have demonstrated that the use of LPCR clearly indicated ΔmtDNA in patients OAT also with small amount of whole seminal samples. A further indication is that the ND5 and ND6 mtDNA region were preferentially associated to deletions with poor quality semen characteristic.



mtDNA *Long* T6-N61 results

PCR product	Long T6 – N61
wt	n. 14 pat.
Δ	n. 30 pat.
(-)	n. 6. pat.
Tot. n. paz.	50

Genotoxicity of terpenes present in wastwater of a citrus transformation factory in bacterial and mammalian cells and effectiveness of photocatalitic degradation.

Marghereth Saverini[a], Giuseppe Avellone[b], Fabio Caradonna[a], Irene Catanzaro[a], Leopoldo Ceraulo[b], Sergio Giuseppe Indelicato[b], Giuseppe Marcì[c], Leonardo Palmisano[c], Giulia Sciandrello[a]
[a] Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo;
[b] Dipartimento di Chimica e Tecnologie Farmaceutiche, Università di Palermo;
[c] Dipartimento di Ingegneria Chimica dei Processi e dei Materiali., Università di Palermo.

The aim of this work was to compare the genotoxic responses of mixtures of terpenes present in wastewaters of a citrus transformation factory with the genotoxicity of the individual compounds. Samplings of wastewater collected before (untreated sample) and past water purification by biological method (treated sample) were analyzed using Solid Phase Micro-extraction (SPME) followed by GC analyses. The chromatograms showed in all effluents the presence of four terpenes: α pinene, β-pinene, 3-carene, D-limonene. The concentrations of terpenes in the untreated sample were 1–3 orders of magnitude higher than in the treated sample.
Genotoxicity was evaluated in the Salmonella reversion assay (Ames test) and in V79 cells by comet assay, by utilizing aqueous solutions the four terpenes at concentrations corresponding at those determined by SPME. In the Ames test, when

tested individually, the four terpenes induced no or only a modest increase of genotoxic effects. On the contrary, the mixtures of terpenes present in untreated sample caused an increase highly significant of the revertants in TA100 strain, in presence of metabolic activation, in comparison to the control. The comet assay showed a significant increase in DNA migration in V79 cells after 1 or 6 h treatment with single or mixed terpenes.
The possibility to photodegrade terpenes by using polycrystalline TiO_2 irradiated with UV light was investigated. Photocatalytic tests carried out on both synthetic and actual aqueous effluents indicated that all terpenes were completely photodegraded, confirming the methodology effectiveness.

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The long way from molecular modeling to biological screening: the discovery of new Hsp90 inhibitors

Anna Maria Almerico[a], Ilenia Abbate[a], Marco Tutone[a], Gaetano Dattolo[a], Francesco Mingoia[b], Antonino Lauria[a]
[a] Dipartimento Farmacochimico, Tossicologico e Biologico - Università di Palermo, Via Archirafi 32, 90123 Palermo, Italy
e-mail: almerico@unipa.it
[b] Istituto per lo Studio dei Materiali Nanostrutturati - CNR, Via Ugo La Malfa 146, 90146, Palermo, Italy

In the last decade the application of computational methodology in the all medicinal chemistry and biology fields has found an amazing development. All these efforts were focused on the searching of new leads possessing a specific bio-molecular target affinity.[1-4]
In this study we focused on the identification of potential Hsp90 inhibitors by (Molecular Docking/Pharmacophore) virtual screening protocols on a subset of compounds from ZincDB. The entire subset was submitted to a two accuracy levels molecular docking screening available in Glide using a Hsp90 structure (2BZ5). Further, the top scored compounds were discriminated using two different sets of pharmacophore hypos (docking and in vacuo), both built using a training set of 49 known inhibitors (BindingDB[5]), belonging to three chemical classes. In the case of Docking-derived pharmacophores, the training set was docked into the active site and the frozen conformations used. In the case of in vacuo derived one, nearly 103 conformers were generated for each training set structure. Thus we recognized a series of derivatives, presenting various core structures, which satisfy each pharmacophore template. The approach of multivariate analysis was applied on an in-house DB of designed compounds. By the two approaches we selected (and synthesized) structures shown in figure. Preliminary analysis of their biological activity showed excellent antiproliferative activities (nanomolar scale) with low toxicity and actually are undergoing in vivo screenings by NCI. Now they are screened to evidence their Hsp90 binding affinity.

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Studies about the effects that ordered structures have on properties of organic substrates.

Francesca D'Anna, Paolo Lo Meo, Salvatore Marullo, Renato Noto, Serena Riela, Paola Vitale.
Dipartimento di Chimica Organica "E. Paternò" Università degli Studi di Palermo.

Supramolecular chemistry studies systems involving aggregates of molecules or ions held together by non-covalent interactions. In this field, initially we have begun to investigate the host-guest interations between cyclodextrins (CDs), natural or modified, and para-substituted nitro benzenes,[1] successively we have pointed out that binary complexes fluorophore-cyclodextrin can act as chiral recognitors.[2] Recently, we have proposed a simple polarimetric method for evaluating the binding constants substrate-CD.[3] The synthesis and characterization of supramolecular hosts constituted by CD and cucurbituril moiety are at this moment in progress.
An other topic relatively to our investigations regarding the supramolecular chemistry is the study of ionic liquids (ILs). They are very studied as an alternative medium to organic conventional solvents. The ILs are constituted by an organic, generally non simmetric, cation and an inorganic, often complex, anion. In terms of supramolecular chemistry ionic liquids are interesting because they present an ordered structure dynamically stable. In this field our studies have the aim to understand as the properties of IL can affect an organic reaction.[4]
More recently our interest has been addressed to synthesis, characterization and utilization of dicationic ionic liquids.[5]
At last, at this moment are in progress studies regarding the design and synthesis of molecules some able to interact with carbon nanotubes for their solubilization, others to interact with DNA.

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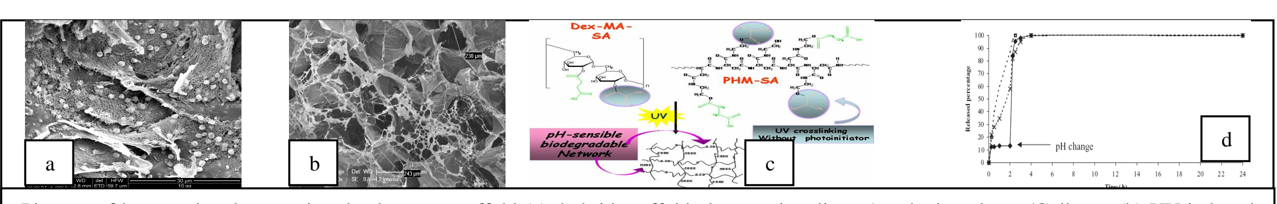
Biomaterials for Biotechnological and Pharmaceutical application

Personale strutturato: G. Pitarresi, F. S. Palumbo, G. Giammona
Dottorandi: F. Calascibetta, C. Fiorica, D. Triolo
Laboratorio di Polimeri Biocompatibili- Dpt di Chimica e Tecnologie Farmaceutiche-Università di Palermo

The Lab of Biocompatible Polymers develops biomaterials for biomedical and pharmaceutical applications. In particular two general classes of materials are produced as hybrid scaffolds made by mixing biocompatible synthetic polymers and natural biomaterials made by extracellular matrix (ECM) polymers.
It is known that more similar by a biological and structural point of view is the material to the ECM, more efficient could be the scaffold for regenerative medicine applications. Our research group engineers hybrid biomaterials as 3D scaffolds, patches or micro-nano particles made by crosslinked ECM's polymers (glycosaminogly-canes, proteins) and synthetic polymers such as polyaminoacids (1). Such hybrid materials exploit both the biological activity of natural polymers and the chemical versatility of biocompatible synthetic polymers. For example regulating the amounts of synthetic polymers we can control biodegradability, mechanical resistance, cellular attachment and spreading. Research interest is also focused onto the chemical functionalization of natural polymers with pendant groups able to induce self assembling or crosslinking producing physical or chemical hydrogels acting as biomimetic scaffolds (2). The gel forming procedures employed are not toxic and allow the safe encapsulation of growth factors and



Pictures of human chondrocytes into hyaluronan scaffold (a), hybrid scaffold glycosaminoglicane/synthetic polymer/Collagen (b) UV induced crosslinking of Dextran/polyaminoacidic hybrid hydrogel employed for Drug Delivery(c); release of ibuprofen from pH sensible hydrogel

cells (3). Moreover the group develops biocompatible devices such as hydrogels, micro and nanoparticles used as intestinal and parenteral drug delivery systems (4,5).

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Localized expression of Strim1, a novel member of the TRIM-containing family, guides the skeletal morphogenetic program of the sea urchin embryo

Vincenzo Cavalieri, Rosa Guarcello, Giovanni Spinelli
Dipartimento di Biologia Cellulare e dello Sviluppo Monroy, Università di Palermo, Viale delle Scienze Edificio 16, 90128 Palermo, Italy
Correspondence to: cavalieri@unipa.it

The building of the skeleton in the indirect developing sea urchin embryo is a complex morphogenetic process that is executed by the Primary Mesenchyme Cells or PMCs (Ettensohn et al, 1997; Wilt 2002). It is well known that the PMCs acquire most of the positional and temporal information from the overlying ectoderm for skeletal initiation and growth (Armstrong 1993; Cavalieri et al, 2003; Röttinger et al, 2008). In this study, we analyze the function of a novel gene, encoding for a tripartite motif-containing (TRIM) protein denoted strim1, that adds up to the list of genes constituting the epithelial-mesenchymal signaling network.
We show that strim1 is expressed in ectoderm regions adjacent to the bilateral clusters of PMCs. Strim1 misexpression causes the number of PMCs to double and leads to skeletal abnormalities. By micromere transplantations, we establish that skeletal defects depend upon strim1 misexpression in ectoderm cells. Reciprocally, knock-down of strim1 function abrogates PMC migration and blocks skeletogenesis. Identical phenotype is shown by chimeras in which strim1 function is blocked selectively in the ectoderm. We also show that clonal expression of strim1 into ectoderm cells from knocked-down embryos restores the correct skeletogenic program. Finally, we report that strim1 triggers the expression of the ectoderm-specific gene pax2/5/8, and the PMC marker sm30 (an ectoderm signaling dependent gene).
We conclude that strim1 function is able to elicit specific gene expression both in ectoderm cells and PMCs to guide the biomineralization during morphogenesis.

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New methodologies in the synthesis and chemical transformation of organic natural products

Gianfranco Fontana and Giuseppe Savona
Dipartimento di Chimica Organica "E. Paternò"

For many years our group has been involved in research programs pertaining several aspects of the natural products chemistry. Many new molecules with a terpenoidic framework have been isolated from a number of plant species and characterized by both modern spectroscopic and chemical methods. Also a lot of new semisynthetic structures have been obtained with the aid of modern selective preparative procedures. Some of these molecules have shown promising biological properties. Interesting examples are the antifeedant activity of some neoclerodane derivatives from Teucrium species (1) and the potential binding activity toward the opioid receptors of some

derivatives of the Salvia genus' metabolites (2,3). In order to further magnify the synthetic tools useful in the transformation of molecules extracted from natural sources, in recent years our group has developed new synthetic procedures based on organometallic reactants and catalysts. For example, the coupling of two electrophilic components – an allylic acetate and an aldehyde – was realized by an In/Pd mediated one-pot procedure (4). Moreover, a palladium catalyzed rearrangement of 3-allyloxy-1,2,4-oxadiazoles to give the corresponding oxadiazolones, a class of syalic acid-related compounds, was successfully developed (5).

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Biochemical and genetic regulation of chromatin remodelling

Davide Corona, Giosalba Burgio, Marianna Collesano, Antonella Ingrassia, Maria Cristina Onorati, Anna Sala, Maria Toto
Dipartimento di Biologia Cellulare e dello Sviluppo & Istituto Telethon Dulbecco, Università degli Studi, di Palermo

Access to eukaryotic DNA in the context of chromatin is granted by enzymatic activities that use the energy of ATP hydrolysis to slide or covalently modify nucleosomes or DNA. These chromatin modifications, occurring without changes in the DNA sequence, set different chromatin functional states and constitute the epigenetic marks of our genome. Despite the wealth of data concerning the mechanisms of action of chromatin remodeling factors and histone modifying enzymes, relatively little is known about how their activities are coordinated and inherited to regulate chromatin structure, gene expression and other nuclear functions. As an independent investigator, I got interested in dissecting the functional network of regulation existing between ATP-dependent remodelers and chromatin factors. ISWI is an evolutionarily conserved nucleosome sliding factor playing essential roles in transcription, DNA replication, and chromosome organization. Using the fruit fly as a model system and a combination of genome wide and bioinformatic approaches we found that ISWI binds genes near their transcription start site affecting nucleosome phasing. Our work shows that higher eukaryote transcription and chromosome organization is regulated genome-wide by the activity of the chromatin remodeling factor ISWI. Furthermore, employing genetic and biochemical approaches, my lab recently found that the activity of ISWI is regulated by several evolutionarily conserved covalent modifiers of chromatin, as well as a number of non-coding RNAs. Our work established that ATP-dependent chromatin remodelers are regulated by a variety of chromatin modifications and factors that escaped previous biochemical characterization. We are currently characterizing some unanticipated nuclear components regulating ISWI function that are involved in stem cell identity and epigenetic memory switch occurring in tissue regeneration.

Bacterial biofilms: a challenge in the discovery of new anti-infective agents

Domenico Schillaci
Dipartimento di Chimica e Tecnologie Farmaceutiche, Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy. E-mail address: dschill@unipa.it
tel. +3909123891915

The ability to form a biofilm, bacterial communities able to grow on surfaces and surrounded by an extracellular polymeric substance (EPS) matrix, is probably the most important virulence factor of staphylococci in the development of the chronic form of some infectious diseases in humans. Furthermore, staphylococcal biofilms are leading causes of device-related infections of medical relevance. It has been observed that S.aureus in biofilms is 100–1000 times less susceptible to antibiotics than equivalent populations of planktonic (single cells) bacteria. Conventional antibiotics can be effective against planktonic cells but currently no therapies that effectively target staphylococcal biofilms exist. Therefore there is an urgent need for novel treatments, strategies and anti-staphylococcal biofilm agents.

Our research group focused on discovering of novel chemical agents of potential therapeutic utility in the treatment of staphylococcal biofilm-associated infections by screening novel compounds (synthetic or natural). In a recent study, the anti-staphylococcal biofilm activity of pyrrolomycins C, D, F1, F2a, F2b, F3 a family of halogenated pyrroles which are naturally produced by Actinosporangium vitaminophylum and of the synthesized related compounds I, II, III were investigated and compared (Schillaci et al, 2010).
We also focused on marine invertebrates as source of new antibiofilm agents and the activity of the 5kDa peptide fraction of the cytosol from coelomocytes of Paracentrotus lividus (sea-urchin) against staphylococcal biofilms was determined (Schillaci et al, 2010).

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Recombinant proteolytic enzymes synthesis. *Cell therapy applications.*

G. Seidita[1-2], M. Salamone[1], F. Bertuzzi[1-3], C. Ricordi[1-4], A. Cuttitta[1-5], S. Rigogliuso[6], A. Nicosia[5], C. Maranto[6] and G. Ghersi[1-6].
[1]ABIEL s.r.l.; [2]Dipartimento di Biopatologia e Biotecnologie Mediche e Forensi, Università di Palermo; [3]Ospedale Niguarda Ca' Granda; [4]Diabetes Research Institute, Miami University; [5]IAMC-CNR, Capo Granitola (TP); [6]Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo.

One of the best examples of successful cell therapy is the transplantation of pancreatic is let. However islet isolation methods are not completely standardized yet, more than half of isolation procedures failed; it is due to variable pancreas condition and to unpredictable enzymatic blend efficiency. Enzymes used for pancreas digestion are purified from Clostridium histolyticum; they have a broad substrate specificity and potent collagenolytic activity compared to vertebrate collagenases. However, a major obstacle in human islet isolation successful is due to the variability in composition and concentration of the collagenases used in the process. Other proteases involved in the procedure are the neutral protease and the termolisine. These play an important role, but they should be considered as a double-edged sword: -accelerates tissue dissociation on one hand, but on other one, result in a decrease islets yield, inducing fragmentation and breakdown. Current parameters characterizing enzymatic blend (due heterogeneity of stocks) are not predictive in pancreas digestion efficiency, consequently enzyme bathes can not be successfully selected in islet isolation purpose.
Our goal is the production of pure enzymes by recombinant DNA technology using synthetic genes obtained through optimization of the nucleotide sequence with the aim to exceed the currently highlighted by commercial enzymes. Than could be used individually under controlled conditions and allow for better reproducibility of the protocols in tissue dissociation; in absence of toxic components.

Evidence for a novel cytoplasmic processing event in ribosome maturation in the sea urchin *Paracentrotus lividus*

Daniele Bellavia and Rainer Barbieri
Dep. Cellular and Developmental Biology "A Monroy", University of Palermo

In this work, we demonstrate the existence of a cytoplasmic processing step, never before described, involving both the pre-ribosomal subunits in the sea urchin Paracentrotus lividus. Northern-blot hybridization, primer extension, S1 mapping experiments and in situ hybridizations allowed us to demonstrate that cytoplasmic processed particles are successively re-imported into the nucleus, where maturation of their RNAs is completed prior to being exported to the cytoplasm. Our findings lead to the proposal of a new model of ribosome maturation and shuttling (1).
Moreover, preliminary data from our laboratory suggest that the maturation pathway we propose in P. lividus may not be unique to the sea urchin, but a common feature in eukaryotes.

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Studies on stem cells: biology of injured stem cells and regenerative potential of microvesicles.

F. Geraci, G. Turturici and G. Sconzo.
Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo

In tissue repair, the injected stem cells localize into inflamed tissues and release soluble factors which induce tissue-resident adult stem cells to participate to tissue replenishment (1). Also mouse mesoangioblast stem cells release several molecules such as FGF2 and MMP2/9 that have proven effective in the regenerative processes occurring in injured tissue, e.g. the infarcted myocardial cells (2) or dystrophic muscular cells. Beside soluble factors, membrane microvesicles (MVs) released from different types of cells including stem cells are increasingly recognized as a new mechanism of cell-to-cell communication (3). Factors enclosed in microvesicles are short-time protected. We found that in vitro mouse mesoangioblast stem cells release active FGF-2 and MMP2/9 through MVs (4). We now found that MMP2/9 synthesis, controlled by NF-kB, is regulated by HSP70 which negatively binds NF-kB and this is supported by HSP70-NF-kB immunoprecipitation analysis. Moreover we found an increased MMP2/9 mRNA transcription in two differently Hsp70 knock down mesoangioblast clones. This clearly indicates that the HSP70 level modulates the level of MMP2/9 synthesis. Consequently, the MMP2/9 released by the MV depend on the amount of HSP70 into mesoangioblasts.

A significant problem in regenerative medicine is that most of the stem cells die when injected in animals to repair damaged tissues. We are focusing this issue by analyzing in vitro the behaviour of cells exposed to H_2O_2, a molecule certainly present in inflamed tissues and able to damage the rushed stem cells.

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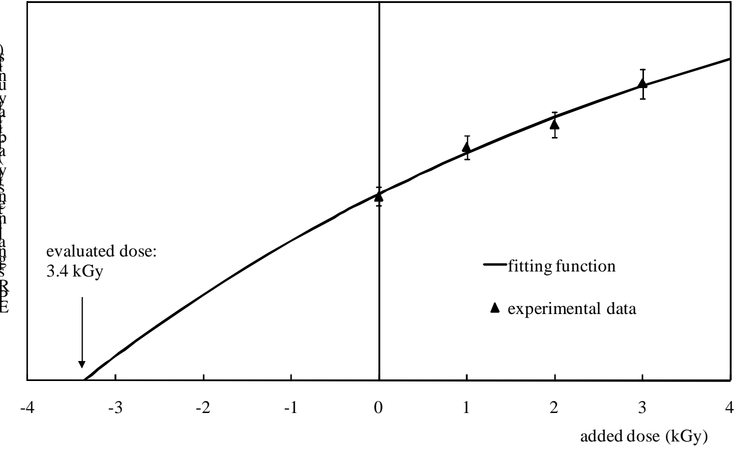
Application of the ESR spectroscopy for the identification of irradiated food containing cellulose and for dose reconstruction

Antonio Bartolotta, Maria Cristina D'Oca
Dipartimento Farmacochimico, Tossicologico e Biologico, Settore di Fisica Applicata, Università di Palermo, via Archirafi 32, 90123 Palermo, Italy
(antonio.bartolotta@unipa.it)

The identification of irradiated food containing cellulose can be achieved by means of the electron spin resonance (ESR) spectroscopy (1), since unirradiated samples give only one single ESR signal, whereas in irradiated samples two satellite



ESR signal intensity vs. added dose for an aliquot taken from nut irradiated at 4 kGy

lines appear, spaced 60 mT each other. Positive identification of these ESR lines ascribable to the cellulose radicals is evidence of irradiation, but their absence does not constitute evidence that the sample is unirradiated. Goals of this work were: i) to extend the ESR spectroscopy as identification method of the irradiation treatment also to animal feed and to foodstuff not yet included in the European Protocol; ii) to use the ESR spectroscopy as a quantitative procedure to evaluate the original dose, using the additive dose method (2, 3). Food samples were irradiated at "original" dose values in the range 1 – 8 kGy. Little aliquots were taken from these samples, the ESR spectrum was recorded, and the peak-to-peak intensity of the satellite lines was measured; each aliquot was re-irradiated with additive doses of 1 kGy each, and the cumulated ESR signal intensity was afterwards measured. Back extrapolation of the best fitting function to the dose axis gives an estimation of the original dose (see figure). Our results show that this method gives an estimation of the original dose within ±30% in all the studied aliquots. A study on the time stability of the ESR signals was carried out, and a procedure was also developed to take into account the signal fading when dose reconstruction is performed after a storage time.

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Histone RNA-binding proteins in the rat brain

Carlo Maria Di Liegro, Patrizia Saladino*, Patrizia Proia**, Gabriella Schiera***, Gemma Palazzolo***, Alessandra Lo Cicero***, and Italia Di Liegro****

*Dipartimento di Biologia Cellulare e dello Sviluppo,
**Dipartimento di Studi Giuridici, Economici, Biomedici, Psicosociopedagogici delle Scienze Motorie e Sportive,
***Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, University of Palermo, Palermo, Italy

Several proteins which bind rat H1° and H3.3 histone mRNAs were enriched from brain cell extracts by chromatography and identified by mass spectrometry. Interestingly, among these factors, heterogeneous nuclear ribonucleoproteins, among which hnRNPs K and A1, were found. By western blot we also evidenced in the purified fraction the CSD-C2 protein (formerly known as PIPPin). CSD-C2 is present both in the nucleus and in the cytoplasm of specific populations of cells, and about one half of the nuclear protein can only be extracted with acid, together with histones. One fraction of this acid-soluble, chromatin-bound CSD-C2 is also sumoylated. The protein is mainly expressed in the adult rat brain, but it is also found in brain tumors and in different cell lines (e.g. NRK and PC12 cells), where its expression is highly increased by induction of differentiation (i.e. in PC12 cells treated with NGF). Since CSD-C2 can also undergo phosphorylation, a modification which affects its RNA-binding ability, we independently mutated some threonine/serine residues of the protein and are now analyzing the effects. In parallel, we are also analyzing possible functional interactions between CSD-C2 and two other proteins recently cloned in our laboratory: PEP-19 and its longer isoform LPI, two camstatins. Finally, since we recently set a protocol to prepare six histidine-tagged recombinant proteins with high specific activity (patent n. PA2009A000029), we used recombinant PIPPin prepared with this method as a bait and isolated a group of PIPPin interactors which are now under investigation.

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Buccal delivery as a new challenge for treatment of motor fluctuations in Parkinson Diseases

L.I. Giannola, V. De Caro, G. Giandalia, M.G. Siragusa, F.M. Sutera, A. L. Scaturro

Dip. Chimica e Tecnologie Farmaceutiche, University of Palermo, Via Archirafi 32, 90123 Palermo Italy

This work was carried out in the frame of the HELP project (AAL, EC-funded) [1] aimed to

manage the progression of Parkinson's Desease (PD) delivering drugs in a controlled, and either continuous or on-demand basis by an application of the so called "IntelliDrug" intraoral device, studied thanks to an FP6-EC grant. The device comprises a medication release mechanism, a built-in intelligence, micro-sensors and micro-actuators, a remote control and can be reloaded in a simple non-invasive way.[2,3]

Buccal delivery could be considered as a good alternative, non-invasive delivery route achieving effective drug plasma and steady state levels very quickly, maintaining these concentrations for prolonged periods. Buccal route is especially advantageous for drugs characterized by an intensive first pass metabolism.[4]

The aptitude of Apomorphine (AM), Ropinirole (RP) and L-Dopa methyl ester (LDME) to permeate the buccal tissue was tested using porcine mucosa mounted on Franz-type diffusion cells as ex vivo model. Drug's permeation was also evaluated in presence of various penetration enhancers and in iontophoretic conditions.[5]

Our experiments demonstrated that while AM was unstable in simulated saliva environment and scarcely cross the membrane, RP and LDME, widely used in treatment of motor fluctuations of PD, well permeate the buccal mucosa reaching adequate plasma levels. Fluxes and permeability coefficients values suggested that buccal mucosa does not appear a limiting step to the drug absorption.

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Studies on secondary metabolites from plants

Sergio Rosselli, Antonella Maggio e Maurizio Bruno

Dipartimento di Chimica Organica "E. Paternò", Università di Palermo, Viale delle Scienze – Parco d'Orleans II ed.17, 90128 Palermo, Italy

Our scientific interest is devoted to the chemistry of natural products from plants. We mainly study plants belonging to the family of Labiatae and Asteraceae, looking for diterpenes and sesquiterpenes occurring in these plants. Isolation and purification of different extracts by several chromatographic techniques, lead to pure compounds, often few milligrams, whose structures are solved using extensive spectroscopic methods. When possible, the reactivity of these compounds is investigated and sometimes chemical modification of functional groups is carried out. Influence of these modifications on the usual biological activity is evaluated in structure-activity relationship studies. Recentely, we investigated the reactivity of germacranolide-type sesquiterpene lactones (SLs) and exploited their biomimetic cyclization into other type of SLs [1]. The influence of the carbocyclic skeleton on the cytotoxic activity has been evaluated [2]. The cytotoxic activity of SLs is related to the presence of an α,β-unsaturated carbonylic moiety, therefore we inserted this functionality in kaurane-diterpenoids and an interesting increase in cytotoxic activity was obtained [3]. Finally, we isolated and solved the structures of several new neoclerodane diterpenoids from Scutellaria species, these compounds showed significant antifeedant activity [4].

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Nonsense polarity, RNA processing and decay in phage f1.

M. Tagliavia, M. Borghesan and M. La Farina

Nonsense polarity in most cases depends on activation of cryptic transcription terminators. We found that the strong polar effect observed in the nonsense polar mutant R4 of phage f1, mapping in the 5' proximal region of gene III, instead depends on enhanced instability of mutant mRNAs, whose pattern can be restored by reduction of RNase E activity. rne -(ts) E. coli strains allowed to explore the mechanisms underlying f1 mRNA processing and degradation.

The major gene III species, a 1.8 Kb long molecule, appeared to be a secondary transcript, whose decay is modulated by a REP, located at its 3' end. The RNA pool of a mutagenized phage unable to form that structure, lacks completely that transcript. Infection of RNaseE-(ts) cells resulted in the accumulation of very large transcripts (3.9-6.4 Kb) and the reduction of the level of the 1.8 Kb transcript. The pool of gene III messages found at non permissive temperature includes transcripts composed uniquely by coding sequences (present also in RNase E+ cells) and mRNAs containing, in their internal portion, the untranslated sequences of the IG region. Thus it appears that in wild type cells these sequences are transcribed and promptly cut by RNase E. Cloning of the IG sequences together with last nt of gene IV into a plasmid, maintaining the natural translation frame, confirmed the presence of RNase E cutting sites into the IG, whose translation in suppressor cells makes them resistant to this ribonuclease. Thus ribosome activity appears to challenge that of the RNase E in mRNA processing.

Organocatalysis and Supported Recyclable Catalysts

Michelangelo Gruttadauria, Francesco Giacalone, Paolo Lo Meo, Serena Riela, Renato Noto
Dipartimento Chimica Organica

The research activity of our group is mainly devoted to two main fields: organocatalysis and supported catalysts. Organocatalysts are metal-free organic compounds of relatively low molecular weight and simple structure capable of promoting a reaction in substoichiometric amount. These molecules have received a paramount interest in the last years because they represent a powerful improvement respect to the most used catalysts for the synthesis of fine chemicals: organometals and enzymes. Moreover, the possibility to support such catalysts, in order to obtain easily recoverable and reusable catalysts, will improve their use for practical applications.

In this context, we have developed the use of simple hydrophobic proline derivatives to be employed in water as mimics of type II aldolase for the stereoselective synthesis of aldols. In parallel, we have obtained new highly stereoselective and recyclable supported organocatalysts to be used in water for the same reaction. Moreover, we have developed a new kind of support which has been called "multilayered covalently supported ionic liquid phase" (mlc-SILP) which has been used as catalyst for the formation of cyclic carbonated and as support for palladium nanoparticles.

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hydrophobically substituted proline

polystyrene-supported proline

mlc-SILP

Examples of materials obtained.

Gordonia sp. SoCg alkB gene confers the ability to degrade and use n-alkanes as carbon source in Gram positive bacteria.

Luca Lo Piccolo[a], *Giuseppe Gallo*[a], *Ruggero la Rosa*[a], *Gianni Renzone*[b], *Andrea Scaloni*[b], *Anna Maria Puglia and Paola Quatrini*[a].
[a]Università di Palermo, Dipartimento di Biologia Cellulare e dello Sviluppo, Viale delle Scienze, Parco d'Orleans II, 90128 Palermo, Italy.
[b]Proteomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, 80147 Naples, Italy.

Gordonia sp. SoCg, a Gram positive strain able to grow on long chain n-alkanes[1], possess a single copy of alkB[2] gene, whose product is required for n-alkane hydroxylation[3]. An analysis of alkB flanking regions revealed five ORFs which were designed as orf1, rubA3, rubA4, rubB and alkU, according to the sequence homology with that of known alk clusters3. In G. sp. SoCg the transcription of these genes was induced by long-chain and solid n-alkanes as revealed by quantitative RT-PCR, and the essential role of alkB in n-alkane degradation was demonstrated by the construction of an alkB disruption mutant strain[3]. The SoCg alkB gene was successfully expressed in Streptomyces coelicolor M145 (M145-AH), and the production of 1-hexadecanol from n-hexadecane oxidation was observed[3].
A differential study of global gene expression of M145-AH cultures was performed, where n-hexadecane (C16) glucose (GLU) and none (NC) were provided as only carbon source, respectively. Proteomic analysis, based on 2D-DIGE and MS procedures, revealed a gradual metabolic adaptation to n-hexadecane utilization, not dissimilar from that one revealed in specialized alkane-degraders[4]. In addition, expression profiles of central carbon metabolism enzymes revealed that the addition of a single gene confers the ability to use recalcitrant pollutants as simple sugars in *Streptomyces*.

Altogether these data, expanding the knowledge on n-alkane bioconversion mechanisms in Gram positive bacteria, could provide new technological platforms for bioremediation studies and strategies.

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Analogues of marine alkaloid nortopsentin: synthesis and antitumor activity

Diana Patrizia, *Barraja Paola, Montalbano Alessandra and Cirrincione Girolamo.*
Dipartimento Farmacochimico Tossicologico e Biologico, Università degli Studi di Palermo, Via Archirafi 32 - 90123, Palermo

Bis(indolyl)alkaloids are recognized as one of the rapidly growing groups of sponge metabolites because of their broad spectrum of biological properties including antimicrobial, antiviral and antitumor activities. Nortopsentins A-C, having a characteristic 2,4-bis(3'-indolyl)imidazole skeleton, showed in vitro cytotoxicity against P388 cells (IC50 1.7-7.8 μg/ml).1 We have reported the synthesis and the antitumor activity of four new series of bis-indolyl-5-membered heterocycles 1-4 in which the imidazole moiety was replaced by

Nortopsentins X = N, Y = NH, Z = CH
1 X = W = CH, Y = N, Z = NR_4;
2 X = S, Y = W = Z = CH;
3 X = O, Y = Z = W = CH;
4 X =W = CH, Y = N, Z = O;
5 X = N, Y = S, W = Z = C;
6 X = W = N, Y = S, Z = CH

7 X = CH; **8** X = N

pyrazole, thiophene, furan and isoxazole rings respectively.2-4 Many other analogues of the marine nortopsentins such as 2,4-bis(3'-indolyl)thiazoles 5, in which the heterocyclic core of the system is constituted by thiazole, have been synthesized. These derivatives possessed strong inhibitory activity against a wide range of human tumor cell lines. In this series replacement of one indole system with a six-membered ring increases the antitumor activity against leukemia and renal cancer cell lines.5 In the attempt of looking for novel antitumor compounds, we thought it was interesting to synthesize new azaanalogues of type 6 in order to verify the possible increase in the antineoplastic activity. We have also synthesized 3-(2-phenyl-1,3-thiazol-4-yl)-1H-indole derivatives of type 7,8.
The antitumor activity and the mechanism of action of nortopsentin heteroanalogues will be discussed.

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PTHrP expression and mesenchymal stem cell differentiation.

<u>Alessandra Longo</u> (1), Rosaria Tinnirello (1), Edda Tobiasch (2), Claudio Luparello (1)
Dipartimento di Biologia Cellulare e dello Sviluppo, Università degli Studi di Palermo
University of Applied Science of Bonn-Rhein-Sieg (D)

It is known that multipotent mesenchymal stem cells (MSC) show the capacity for multilineage mesenchymal differentiation. In particular, MSC can differentiate towards osteoblasts, adypocites and chondroblasts using in vitro tissue culture-differentiating conditions [1]. Adipose tissue is an accessible and rich source of adult MSC (AMSC) which can be isolated from liposuction specimens and cultured [2]. Adipogenic and osteogenic differentiation can be obtained after 28 days, and confirmed using staining techniques and checking the expression of specific genes [3]. Although the differentiation of AMSC from adipose tissue into the adipogenic and osteogenic phenotypes are standard procedures, the underlying molecular mechanisms and pathways are still poorly understood. To better understand the potentials of AMSC in cellular therapy and in tissue engineering, it is important to study the initial commitment and differentiation of these cells, by the identification of novel regulatory genes and factors involved in the processes of early adipogenesis and osteogenesis.
Parathyroid hormone-related peptide (PTHrP) is a regulator of cellular proliferation, differentiation and apoptosis in many cell types [4, 5]. To search for stemness/differentiation markers, we aimed to study PTHrP gene expression, with particular interest to its splicing isoforms, to highlight when the chronological expression of the different variants, if any, starts and whether it varies over time, both in control stem cells and in those induced to differentiation.

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Chemistry and bioactivity of natural products

<u>Di Stefano Vita</u> and Pitonzo Rosa.
Dipartimento di Chimica e Tecnologie Farmaceutiche, Facoltà di Farmacia
Via Archirafi 32, 90123 Palermo

Natural products have been the most successful source of drugs ever. Historically, the most important natural sources have been plants. Research has followed two main lines: ethnopharmacology and toxicology that have produced many valuable drugs and are likely to continue to produce lead compounds.

One of the main objectives of chemistry of natural compounds is the identification of biologically active secondary metabolites such as terpenoids, flavonoids, alkaloids, polyphenols, sterols, unsaturated fatty acids, found in vegetable matrices.
Traditionally, the natural sources investigated in our laboratory were mostly plants, resins and essential oils, in order to assess the pharmacological activities of isolated chemical constituents.
The plant matrices are subjected to extraction procedures (liquid-liquid extraction, steam distillation, soxhlet and SPE) spectrophotometric analysis (UV, IR, NMR), chromatographic treatments (TLC, flash column chromatography, HPLC and GC).
The conducted phytochemical investigations have included: oleogum resins produced by different types of trees of the genus *Boswellia spp*. and their essential oils, *manna* produced from cultivars of *Fraxinus angustifolia* and *Fraxinus ornus*, oranges and grapefruits of the species *Citrus sinensis* (L) Osb., *Citrus aurantium* subsp. Myrtifolia, *Citrus paradisi* , aerial parts of *Athamanta sicula* L. and *Euphorbia bivonae* Steudel, resins and aerial parts of *Dracaena draco* L.. The chemical constituents isolated from plant material were tested for their antimicrobial activity, antioxidant, antiproliferative and antibiofilm properties.

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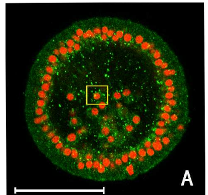
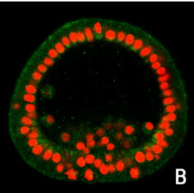
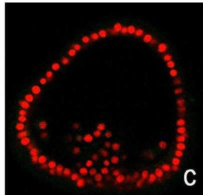
Cadmium induces autophagy during development of *Paracentrotus lividus* embryos

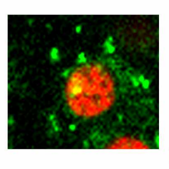
Agnello Maria, Chiarelli Roberto, Morici Giovanni and Roccheri Maria Carmela
Dipartimento di Biologia Cellulare e dello Sviluppo, Viale delle Scienze, Ed. 16, Università degli Studi di Palermo.

P. lividus embryos adopt different defense strategies against cadmium stress such as synthesis of hsps and/or activation of apoptosis [Roccheri et al., 2004; Agnello et al., 2007; Agnello and Roccheri 2010]. Here we show that this model system adopts autophagy as an additional strategy to safeguard the developmental program and we show an interesting relationship with apoptosis. It is known that autophagy can be a survival mechanism, but also a device of PCD-II. The use of several investigation methods are suitable for autophagy detection (Kelekar, 2005).
We found that in *P.lividus* embryos autophagic processes occur, at basal levels during physiological development and at greater levels after 1mM $CdCl_2$ treatment.
By Acridine Orange vital staining on whole embryos and Confocal Laser Scanning Microscopy (CLSM) we detected the acidic vescicular organelles (AVOs) or autophagolisosomes rate. In order to confirm this data we employed an antibody against LC3 protein, a specific marker of autophagy, both through Western blotting and immunofluorescence/CLSM analysis. Concomitantly bafilomycin A1 has been used as a late inhibitor of autophagy.
Furthermore we have detect the temporal and functional autophagy/apoptosis relationship analyzing, in the presence of the autophagy









Immunofluorescence analysis of LC3 protein in whole embryos, after 18 hours of development. Equatorial optical sections captured by CLSM. In green LC3 protein, in red nuclei stained with propidium iodide. A) Cadmium treated embryo B); Control embryo; C) Negative control; D) Enlargement of a particular of A. Bar=50µm

inhibitor, the rate of apoptotic fragmented DNA through the in situ TUNEL assay.
On the basis of these results we hypothesize that autophagy can be adopted in sea urchin embryos as a cell survival defence strategy for the safeguard of the development program or for the ATP supply in apoptotic process.

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Mass spectrometry applications on supramolecular chemistry and biomedical and alimentary analysis.

Avellone G.[1]*, Agozzino P.*[1]*, Bongiorno D.* [1,2]*, Ceraulo L.* [1,2]*, Filizzola F.* [1]*, Indelicato S.* [1,2]*, Indelicato S.G.* [1]
[1] Dip. di Chimica e Tecnologie Farmaceutiche, Università degli studi di Palermo, via Archirafi 34, 90123, Palermo
[2] Centro Grandi Apparecchiature, UniNetLab, Università degli studi di Palermo, via F. Marini 14, 90128, Palermo

The workgroup has been dealing for many years with the development of several methods involving mass spectrometry. The fields of applications are quite large and range from biomedical analytical applications, to food components characterization for traceability and quality assessment. Structures elucidation, reaction pathways determination, supramolecular chemistry in gas phase are other important topics of our research group.
Some recent developments in the field of biomedical analytical applications, food and supramolecular chemistry will be briefly described:
Determination of halogenated anesthetics in urines.
Methods for the determination of a great number of toxins, micotoxins, pesticides, VOCS or other contaminants (natural or artificially added) both in food and in water or beverages.
Characterization and quality control of food and beverages typical of our region.
Surfactant self assembly in gas phase.

Chromatin dynamics during sea urchin embryogenesis: effects on the neural alpha tubulin PlTα2 gene expression

Marco Emanuele, Salvatore Costa, Maria A. Ragusa, Fabrizio Gianguzza
Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo, viale delle Scienze Ed. 16, 90128, Palermo, Italy

Expression of PlTα2 gene during sea urchin P. lividus development, is spatially confined to the neural territory and temporally activated from the blastula stage. To evaluate a possible involvement of chromatin modifications in regulation of PlTα2 gene expression we first searched for DNaseI hypersentive sites. We found four sites localized in the introns of the gene, when we used chromatin extracted from embryos at gastrula stage but not from morula stage. This result suggests a possible functional role of the introns in the activation of the expression of PlTα2 gene. Moreover, we used specific antibodies for RNA polymerase II and for different modified form of lysine 9, lysine 27 and lysine 4 of the H3 histone in quantitative Chromatin Immuno Precipitation experiments to emphasize the different state of chromatin during embryo development. Our analysis shows high H3K9 acetylation and H3K4 trimethylation degree in nucleosomes located at α2 promoter region in P. lividus embryos at gastrula stage, when the PlTα2 expression level is high. This observation agrees with conventional positive role assumed by these post-translational modifications in chromatin remodeling leading to increase promoter accessibility. Furthermore ChIP analysis shows also high H3K27 dimethylation degree during all development stage but ReChIP analysis shows no co-occupancy of this modification with RNA polymerase II in promoter region in embryos at gastrula stage. This observation is consistent with the hypothesis of a general repressive role of this modification in the non-neural territory of the embryo.

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Heterocyclic Chemistry: from Basic Research to Applications in Materials Science and Medicinal Chemistry

Silvestre Buscemi, Andrea Pace, Antonio Palumbo Piccionello, Ivana Pibiri, and Nicolò Vivona
Dipartimento di Chimica Organica "E. Paternò", Università degli Studi di Palermo, Viale delle Scienze - Parco d'Orleans II, Ed. 17, I-90128 Palermo, Italy

Heterocycles represent an important class of organic compounds with a variety of applications as bioactive molecules and new materials.
Our research mainly focuses on the chemistry of heterocyclic compounds from their synthesis [1] to the study of their thermal [2] and photochemical [3] reactivity and has recently developed towards their applications in materials science [4] and medicinal chemistry [5].
Studied systems include both five-, and six-membered heteroaromatic compounds, whose chemistry has been investigated in solution and in constrained media (zeolites) which can mimic enzyme–like environments.
Moreover, modern methodologies have been developed for the synthesis of functional heterocycles, including mono- and poly-fluorinated systems, which have found applications as Ionic Liquids (ILs), Ionic Liquid Crystals (ILCs), Fluorescent Sensors for detecting heavy metal ions, Hydrogelators, Antibacterials, DNA Groove Binders with anticancer activity, and Molecular Probes for non-invasive diagnostic imaging.
Collaborations in current multidisciplinary projects involve physical, computational, inorganic and medicinal chemists, microbiologists, biochemists, genetists, physics and radiologists.

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Indicaxanthin up-regulates cox-2 gene and increases cyclopentenone levels in lps-activated raw264.7 macrophages.

M. Allegra, L. Tesoriere, F. Angileri, C. Gentile, A.M. Pintaudi, M.A. Livrea
Dipartimento Farmacochimico, Tossicologico e Biologico, Università di Palermo.

Introduction

Cyclooxygenease-2 (COX-2) is not simply a pro-inflammatory enzyme that generates a PGE2-dominated eicosanoid profile during the development of inflammation. Indeed, the enzyme even counter-regulates the process by generating an alternate set of prostaglandins such as the cyclopentenones throughout the resolution phase (1). The expression of COX-2 is regulated by several transcription factors and significantly depends on the endocellular redox potential. Indicaxanthin is a redox-active betalain pigment recently acknowledged as a free-radical scavenger (2). This work has evaluated the effects of indicaxanthin on COX-2 gene expression, as well as on the PGE2 and 15d-PGJ2 production, in LPS-activated RAW264.7 macrophages.

Materials and Methods

Indicaxanthin was isolated from cactus pear fruits as previously described (2). Conditions for RAW264.7 cell culturing, LPS stimulation, COX-2 mRNA, PGE2 and 15d-PGJ2 detection were according to Pan et al. (3).

Results and Conclusions

Pre-treatment with indicaxanthin (50, 75, 100 µM) of LPS (1µg/ml)-activated RAW264.7 macrophages increased COX-2 gene expression in a concentration-dependent manner (4.25±0.35; 10.54±0.98; 25.47±1.98 fold increase respectively, vs LPS-activated cells, n=3). Interestingly, when LPS-activated macrophages were pre-incubated with indicaxanthin at 100µM, PGE2 levels were reduced by 50±4.3% (n=3), while the 15d-PGJ2 ones were increased by 562±23% (n=3).
In conclusion, indicaxanthin has a unique ability to

drive the eicosanoid synthesis towards the generation of anti-inflammatory prostaglandins.

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Bypass of G1 arrest induced by DNMT1 post-transcriptional silencing triggers aneuploidy in human cells.

Barra Viviana, Lentini Laura and Di Leonardo Aldo
Dipartimento di Biologia cellulare e dello Sviluppo "A.Monroy"

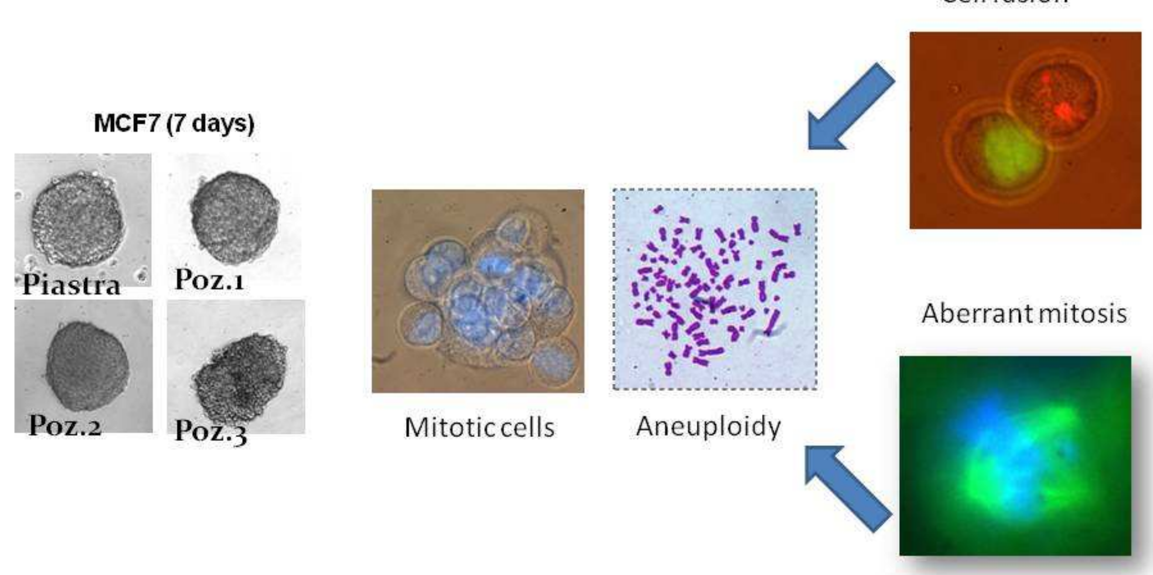
Aneuploidy is a major source of genomic instability in cancer, resulting from chromosome segregation errors caused by defects in genes controlling correct mitotic spindle assembly, centrosome duplication and cell cycle checkpoints. Interestingly in aneuploid cells some of these genes, although not mutated, were underexpressed suggesting the involvement of epigenetic alterations. DNA methylation and histone modifications are the main epigenetic modifications occurring in cells. DNA methyl-transferase 1 (Dnmt1) is known to restore DNA methylation patterns during cell divisions. We investigated the effects of DNMT1 silencing by RNA-interference on the generation of aneuploidy in primary human fibroblasts (IMR90) and stable near-diploid human tumor cells (HCT116). Dnmt1 depletion induced aneuploidy in addition to cell proliferation delay in HCT116 cells and transient G1 arrest in IMR90 cells. IMR90-siDNTM1 cells showed increased levels of the TP53 tumor suppressor. Moreover, cells where DNMT1 and TP53 were simultaneously silenced entered the cell cycle, suggesting TP53 as likely responsible for the observed G1 arrest. DNMT1 downregulation was also associated to global DNA demethylation in HCT116 cells and to partially decondensed pericentromeric chromatin in IMR90 cells. Our results suggest that Dnmt1 depletion triggers a cell cycle arrest pathway mediated by TP53 in IMR90 cells, whose dysfunction induces aneuploidy likely affecting the correct chromosome segregation by altering pericentromeric structure.

Poster

Identification of mechanism(s) leading to hyperdiploidy in progenitor tumor cells derived from MCF7 breast cancer cells

Laura Lentini, Viviana Barra and Aldo Di Leonardo

Stem cells are a minor population of mostly resting cells defined by their long life, high clonogenicity, self-replicating potential, plasticity, and drug resistance (Finn, 2008). Cells with these properties have been identified in various normal and cancerous human tissues (Wicha, 2006), as well as in several long-term tumor cell lines (Setoguchi, 2004). We have some preliminary data indicating that cells isolated from MCF7 line divide slowly and form spheres, both features of progenitors tumor cells, when grown in ultralow adherent plates and in absence of serum. Furthermore, these features were associated to two distinct populations characterized by different content in terms of number of chromosomes. We hypothesize that there are mechanisms that lead to the formation of hyperdiploid cells starting from progenitors cells with a near-diploid karyotype. We are investigating which genes might be involved in this process and if symmetric or asymmetric division depending by altered mitotic genes expression. Moreover we supposed that cell-cell fusion could be a mechanism that lead to hyperdiploidy. To understand whether changes in ploidy occurring in progenitor cells might be the result of cell fusion, we transfected cells from MCF7-sphere with two vectors encoding H2B-GFP (green) and H2B-RFP-ruby (red) to stain chromosomes. Cell were co-cultured and fusion occurrence was evaluated by fluorescence microscopy detecting nuclei with both types of fluorescence, indicating fusion as possible mechanism to generate hyperdiploid cells.



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Effects of different Ras mutations on HT-29 colorectal carcinoma cell line.

Fabrizio Miranda, Mario La Farina, Ida Albanese
Department of cellular and developmental biology, University of Palermo, Palermo, Italy

The Ras oncogene is mutated in about 30% of the human tumors and its mutations are almost always point mutations concerning codon 12, 13 and 61. These mutations cause in the proteins a reduced GTPase activity, so that they become costitutively active. In human cells there are threee main isoforms of Ras (H, K, N-Ras) which can trigger alternative pathways of signal transduction. In order to investigate the effects of expression of different oncogenic Ras isoforms in colorectal carcinoma cells (HT-29), we obtained stable clones of HT-29 cells transfected with cDNAs codifying H-RasG12V and K-RasG12V called respectively H12 and K12 and K-RasG13D called K13, under the control of an hormone-inducible promoter. We observed changes in shape and growth rate when mutated Ras was espressed. We then focused on the clone H12 and we have analized their cell cycle and apoptotic rate by FACS analysis and Annexin V staining. The levels of several regulator of the cell cycle have also been analyzed with particular attention to the p21 gene expression. We have

finally performed treatments with inhibitors of different proteins acting downstream of Ras to better understand which pathway is involved in the morphological and molecular changes observed. Our results show that specific mutations of different Ras isoforms have different effects on morphology and gene expression in HT-29. H-RasG12V expression, in particular, seems to determine apoptosis through caspase activation mediated by p53 independent p21 expression.

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Tryptophan metabolism in Streptomyces coelicolor.

F. Zummo, A. Giardina, R. Alduina, P. Spinelli and A.M.Puglia.
Dipartimento di Biologia Cellulare e dello Sviluppo, Università degli Studi di Palermo

In Streptomyces coelicolor tryptophan biosynthetic genes (trp) are spread on the chromosome (Merino et al., 2008). They are not feedback-regulated and their expression seems to be growth-phase dependent (Hu et al., 1999). On the other hand only a few genes involved in tryptophan catabolism have been identified.
This study has been focused on the role of single-copy genes belonging to the trpCXBA cluster. trpA and trpB genes encode the two subunits of the tryptophan synthetase. Surprisingly, qRT-PCR analysis suggests that they are differentially expressed. S1 mapping analysis reveals two transcription start sites upstream of trpA and trpB indicating that their expression is finely regulated.
Moreover, we isolated and characterized a three-gene cluster (kyn) involved in tryptophan catabolism via anthranilate production. kynA, kynB and kynU gene expression increases in the presence of tryptophan.
In Streptomyces coelicolor, tryptophan is one of the precursors of the calcium dependent antibiotic (CDA) (Kim et al., 2004). In order to elucidate the role of tryptophan metabolism we constructed trpA and trpB null and overexpressing mutant strains. Knockout mutants show tryptophan auxotrophy and produce less antibiotic than wt strain; on the contrary, trpA and trpB overexpression mutants exhibit an early CDA production. Furthermore, kynU knockout mutant shows an increase in CDA production.
Our results provide new insight into tryptophan metabolism and regulation in S. coelicolor.

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Molecular approaches to elucidate the early response of P. lividus embryos to sublethal Cadmium exposition

Maria A. Ragusa, Marco Gianguzza, Maria Carmela Roccheri, Fabrizio Gianguzza*
Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo, viale delle Scienze Ed. 16, 90128, Palermo, Italy
*address for correspondence: marag@unipa.it

Environmental pollution of heavy metals is very abundant nowadays from industry, chemicals, old paints, and pipes or resulting from previous contaminants accumulating in the food chain. Cadmium (Cd) is a toxic heavy metal contaminating coastal environments, especially the estuary of polluted areas.
The toxic effects of high Cd concentration have been studied in embryos of marine invertebrates. In particular, Paracentrotus lividus sea urchin embryos developed in 1mM CdCl2 undergo to development arrest or severe malformations. Cd is able to activate different molecular responses (HSPs synthesis, autophagic or apoptotic processes) that can allow embryo survival.
This research is aimed to study the early defense strategies activated by P. lividus 30 hours phenotypically normal embryos, in response to exposition to lower doses of Cd (100uM CdCl2), analyzing the induced transcriptome and comparing it to that of control untreated embryos by RDA technique, that synergically exploits the characteristics of both subtractive hybridization and PCR.
Our preliminary results suggest the activation of a typical defensome response, they show in fact the up regulation of four metallothionein genes,

selenophosphate synthase gene and genes coding for different oxidoreductases. Moreover, membrane transport protein coding genes are activated, like Na/K ATPase, ABC family members, and signal transduction kinases, like BMPR1, TAK1, histidine kinase. To confirm these results, we are checking the variations in gene expression levels by quantitative real-time RT-PCR.

Computational prediction of the Helitron-N2 (HeN2) transposable element in the strim1 locus of the Paracentrotus lividus genome.

Rosa Guarcello, Giovanni Spinelli and Vincenzo Cavalieri
Dipartimento di Biologia Cellulare e dello Sviluppo A. Monroy, Università di Palermo, Viale delle Scienze Edificio 16, 90128 Palermo, Italy
Correspondence to: cavalieri@unipa.it

Key words: transposable element Helitron-N2, TRIM, sea urchin

Transposons constitute a significant component of the eukaryotic genome. Among others, Helitrons represent a novel major class of eukaryotic transposons, and are fundamentally different from classical ones in terms of their structure and mechanism of transposition [1]. In particular, Helitrons constitute ~1% of the sea urchin genome [2]. By a in silico approach focused on the genome of the Mediterranean sea urchin Paracentrotus lividus, we have predicted regions of high sequence identity to a Helitron-N2 (HeN2) element in the strim1 locus. Of interest, HeN2 sequences lies within the 5' non coding region of the strim1a and strim1b genes, spanning from -105 to -2255 (the start codon ATG of strim1 is defined as +1). As reported in other species, HeN2 is a non autonomous transposable element, lacking the sequences coding for the DNA polymerase and Helicase enzymes. A deep computational inspection of the HeN2 element revealed its structure. It consists of two terminal palindromic sequences (U5 and U3), and eleven tandemly arranged 155bp-long direct repeats (DR1-11). It is well known that the TRIM-containing gene family, to which strim1 belongs, is widely complex and heterogeneous due to the combination of exon shuffling, duplication and/or deletion events that likely occurred during evolution [3]. Interestingly, Helitrons are a formidable evolutionary tool owing to their ability to capture host genes [4] and transposition plays important roles in the evolution of duplicated genes [5]. Therefore our finding could suggest a mechanism for the evolution of the TRIM multigenic family.

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Effects of an inflammatory mediator as H_2O_2 on mesoangioblast stem cells

Turturici G, Cavallaro A, Barone F, Di Trapani M, Sconzo G, Geraci F.
Dipartimento di Biologia Cellulare e dello Sviluppo

Different type of stem cells, when injected or engrafted in a damaged tissue, may contribute to tissue repair, but often many cells die for the adverse microenvironment of inflamed tissues. To explored the causes of this phenomenon we used mouse mesoangioblast stem cells which are known to promote the recovery and improve the damaged tissues when injected into animal models (e.g. with myocardial infarction or with experimental muscular dystrophy). We analyzed the mesoangioblast behaviour "in vitro" by submitting them to H_2O_2 effect at two concentrations similar to that found in inflamed tissues (400 μM or 100 μM) and observing their recovery (rec.) up to 8 days. The results showed that 400 μM H_2O_2 treatment for 24h blocks mesoangioblast growth and 50% of cells die during this period. In the first 2 days of rec. there is the G_2/M cell cycle arrest and also during the first 2 days of rec with the simultaneous activation of autophagy that decreases and then disappears in 5 days of rec. The apoptosis is activated after treatment at low levels reaching a peak at 5 days when autophagy is back to normal levels. Cells die for apoptosis and necrosis also a

8 days of rec. as observed by annexin assay. Treatment with H_2O_2 at 100 µM don't block cell growth, induce G_2/M cell cycle arrest only during 24h of treatment. Also activates the apoptotic pathway at 5 days of rec, and like 400 µM a part of cells continue to die up to 8 days of rec.

Indicaxanthin inhibitory effects on mouse ileal contractility: analysis of the mechanism of action.

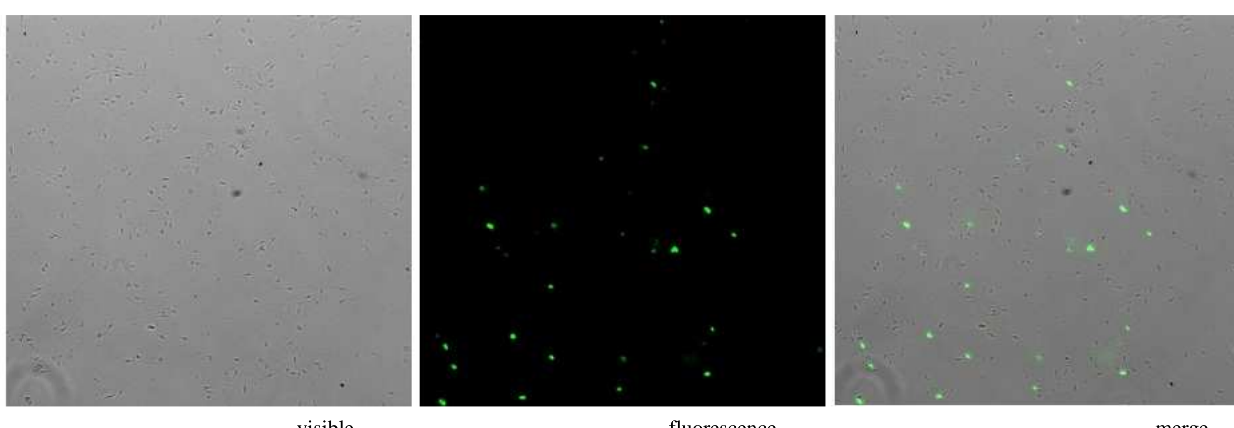
Sara Baldassano[a], Alessandra Rotondo[a], Antonella Amato[a], Rosa Serio[a], Maria Antonietta Livrea[b], Luisa Tesoriere[b] and Flavia Mulè[a].
[a]Dipartimento di Biologia cellulare e dello Sviluppo,
[b]Dipartimento Farmacochimico Tossicologico Biologico, Università di Palermo, 90128 Palermo Italy.

The purpose of the present study was to investigate the mechanism of action responsible for the spasmolytic effects induced by indicaxanthin, the yellow betalain pigment abundant in Opuntia ficus indica fruit, on the intestinal contractility. Using organ bath technique to record the mechanical activity of mouse ileum longitudinal muscle, we observed that indicaxanthin action was not affected by indomethacin, non-selective inhibitor of cycloxygenase, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, selective inhibitor of nitric oxide-dependent guanylyl cyclase, 2'5'dideoxyadenosine, adenylyl cyclase inhibitor, and zaprinast, a selective inhibitor of the cGMP phosphodiesterase isoenzyme. It was significantly reduced in the presence of 3-isobutyl-1-methylxanthine (IBMX), a non selective inhibitor of phosphodiesterases (PDEs). Indicaxanthin and IBMX significantly reduced the carbachol-evoked contractions and the joint application of both drugs did not produce any additive effect. Indicaxanthin and IBMX increased the inhibitory effects of forskolin, an adenylyl cyclase activator and the joint application of both drugs did not produce any additive effect. Indicaxanthin, contrarily to IBMX, did not affect the inhibitory action of sodium nitroprusside, a soluble guanylyl cyclase activator. ELISA test showed that indicaxanthin increased the cAMP content of mouse ileal muscle. The present results suggest that indicaxanthin from Opuntia ficus indica is able to reduce the contractility of ileal longitudinal muscle by inhibition of PDEs and consequent increase of intracellular cAMP.

Evaluation of DNA integrity and quality sperm comparing different freezing protocol, in oligoastenospermic patients.

L. Bosco, G. Ruvolo, T. Cirrito, G. Faulisi, R. Gelo, G. Taormina and M.C. Roccheri
Dipartimento di Biologia Cellulare e dello Sviluppo, A.Monroy, viale delle Scienze ed.16 Palermo

Sperm cryopreservation, is used in the case of preservation of male fertility before radiotherapy and/or chemotherapy which may lead to testicular failure or ejaculatory dysfunction. Our interest has been to verify the effect of high cooling rate of vitrification and the toxic effect of higher concentration of cryoprotectants (CPAs). Each ejaculate was prepared by swim-up and divided for: traditional freezing protocol with the standard used CPAs. Test yolk buffer (TYB), vitrification with (CPAs+) or without (CPAs-) cryoprotectants. DNA



visible fluorescence merge

Sperm apoptosis evaluation by TUNEL assay. Images of human sperm observed through fluorescence microscopy.

fragmentation was investigated using TUNEL assay in situ. We found a significant sperm motility reduction comparing fresh samples with TYB (47% vs 24%). Absence of sperm motility was found in CPAs-, and lower motility rate was found in CPAs+ if compared with TYB (4.6% vs 24 %). Higher significant DNA fragmentation was found in CPAs-, CPAs+ and TYB compared with fresh samples (respectively 52.2%, 44.8 %, 46.1 and 21.6). No difference in terms of DNA fragmentation was found between the three different freezing protocols. We found that sperm motility and DNA integrity is affected by freezing procedure. Motility seems to be completely affected in CPAs-vitrification procedure. The DNA integrity of CPAs+/CPAs- vitrified sperm is comparable with that shown by conventional freezing protocol spermatozoa. Sperm can be used in ICSI fertilization procedure. Vitrification can be used as a quick and simple method. The use of vitrification with CPAa allow to obtain motile sperms. after thawing.

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DNA extraction from phytoseiid specimens preserved on a glass slide and molecular diagnostic

Barbara Salerno and Elena Carra
Dipartimento di Biologia Cellulare e dello Sviluppo
Università di Palermo

This study focuses on the diagnostic of seven species of mite belonging to the family of Phytoseiidae; very important biocontrol agents of phytophagous mites. However , they are morphologically very similar and specific diagnostic is very difficult in fact using the traditional identification methods it is uncertain to identify a species especially when Authors use different characters, very often not stable in the time. In the 60's M.me Athias-Henriot took into consideration the insemination apparatus that is considered a stable character in the time. According to her, the shape of this apparatus could be considered a major criterion for genus distinction. The aim of the present study is to establish, using molecular investigation, which theories is more correct. DNA were extracted from specimens preserved on a glass slide by three different methods, the organic (phenol-chloroform) Method, the Chelex® 100 extraction procedure and the DNA extraction by a DNneasy Tissue Kit.
Phenol-chloroform extraction takes a relatively long period of time (5-6 days), but it produces consistent results. The modified Chelex technique involves less time and work (only 2 hours) and doesn't require toxic organic solvents, the Qiagen kit did not allow extraction from mites mounted on glass slide 20 years ago. Molecular result (12S rRNA and RAPD-PCR) showed a clear difference between the species.

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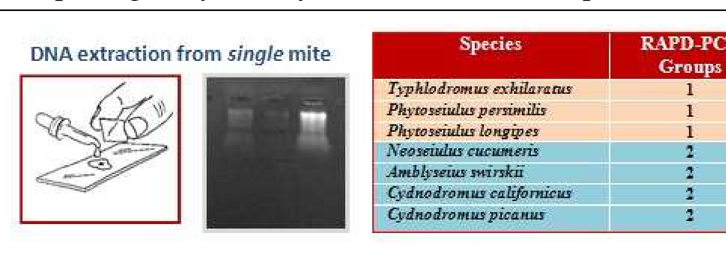
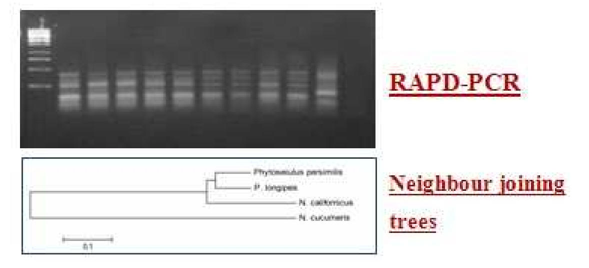
Extracellular membrane vesicle shedding and the blood-brain barrier

Gabriella Schiera, Patrizia Proia**, Alessandra Lo Cicero*, Gemma Palazzolo*, Erika Gucciardo*, Evelina Ferrantelli*, Carlo Maria Di Liegro***, Giovanni Savettieri*, and Italia Di Liegro**
*Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, **Dipartimento di Studi Giuridici, Economici, Biomedici, Psicosociopedagogici delle Scienze Motorie e Sportive, ***Dipartimento di Biologia Cellulare e dello Sviluppo, University of Palermo, Palermo, Italy

The blood-brain barrier (BBB) is formed by brain capillary endothelial cells (BCECs), under the



Species	RAPD-PCR Groups
Typhlodromus exhilaratus	1
Phytoseiulus persimilis	1
Phytoseiulus longipes	1
Neoseiulus cucumeris	2
Amblyseius swirskii	2
Cydnodromus californicus	2
Cydnodromus picanus	2



effects of neurons, pericytes, and glial cells. BCEC form tight junctions (TJ), that seal them together. TJs also contain occludin, which undergoes post-translational modification before being associated with TJs: it is considered a marker of TJ maturation and of BBB stabilization. We set an in vitro model of BBB to study the role of neurons and astrocytes in the establishment of the barrier (1-2). The paracellular flux of compounds unable to cross the BBB in vivo (e.g. dopamine and sucrose) significantly decreased while the transendothelial electrical resistance (TEER) increased when BCECs were co-cultured with astrocytes and neurons (2). Moreover, BCECs cultured with astrocytes and/or neurons, or fed with conditioned media, produce more occludin and tend to localize it at the cell periphery, thus suggesting formation of TJs and confirming BBB formation. Since we also discovered that oligodendroglioma cells shed extracellular vesicles (3) which contain angiogenic and proapoptotic factors, we investigated whether also neurons and/or astrocytes can influence BCEC behaviour by releasing vesicles. The results demonstrated that all kinds of brain cells release extracellular vesicles, that contain angiogenic factors, such as FGF-2 and VEGF (4-5). We are now investigating the possibility that the BBB model can be used to study molecular events that result in BBB damage, in some pathological conditions, such as, for example, multiple sclerosis (6).

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Tissue Engineering Biopolimers

S. Rigogliuso[1], F.Carfì Pavia[2], G.Lo Re[2], M.A.Sabatino,[2] F.Segatti[3], V.Brucato[2], R.Scaffaro[2], C.Dispensa[2], M. Epifanio[1], E. Bonafede[1] and G.Ghersi.[1]

[1]Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo.
[2] Dipartimento di Ingegneria Industriale (D.IN.I) Università di Palermo.
[3] LIMA Lto, SpA. Udine

Tissue engineering is a multidisciplinary research area focused on tissue regeneration. Development of biocompatible devices has brought the world of substitutive medicine in what considered the greatest therapeutic revolution of our times. The main challenge of biomaterials is not only related to "tolerance-biocompatibility" by the body but also in its functionality. In this work we evaluated biocompatibility of different polymers types; this activity was carried out in collaboration both LIMA Lto and D.IN.I Palermo's University. We tested "scaffolds," characterized by different chemical/physical/structural parameters. HA-PLLA, PLA-PEG and PLLA, scaffolds, designed respectively to replace mineralized structures and "soft" tissue, supporting vascularization of biocompatible polymers. Since supply of nutrients and oxygen are diffusion phenomena insufficient vascularization can lead to the death of tissue. Use of angiogenic scaffolds could solve this problem and improve the success of the system. Another area of our interest is the controlled release of drugs in the body, using nanocarriers. We are testing biocompatibility of PVP hydrogels, that once properly functionalized and loaded, can be used for biomedical applications. We test both the non-cytotoxicity of "scaffolds" such as cell adhesion and proliferation inside them; even for the hydrogels, apoptotic tests were performed. Our data shown that both, scaffolds and PVP hydrogels, do not induce cell toxicity, so they can be considered suitable for the application in designed aimed.

Babesia bigemina surface antigens: an overview

Blanda[1] V., Albanese[1] I., La Farina[1] M., Sireci[3] G., Agnone[3] A., Torina[2] A.

1 University of Palermo, Dipartimento di Biologia Cellulare e dello Sviluppo
2 National Reference Centre for Anaplasma, Babesia, Rickettsia and Theileria (CRABaRT), Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri"
3 University of Palermo, Dipartimento di Biopatologia e Metodologie Biomediche

Cattle babesiosis is a tick-borne disease transmitted by haemoparasites such as Babesia bovis and B.bigemina, affecting cattle in tropical and subtropical areas. The study was addressed to the molecular characterization of B.bigemina strains isolated from infected animals, with attention to the genes that codify for surface antigens, putative candidates for vaccine and diagnostic tools development. One of the molecules potentially involved in the erythrocyte invasion by B.bigemina is the Apical Membrane Antigen-1 (AMA-1), an

apically located protein that is shared by many Apicomplexa. This study provides useful information on AMA-1 gene from Italian strains of the parasite B.bigemina and some predictive information related to the protein obtained by bioinformatics tools. The rhoptry associated protein-1 (rap-1) is considered one of the most interesting vaccine candidate against bovine babesiosis. Herein the sequence of the rap-1b gene from a Italian strain of the B.bigemina is reported for the first time. Surface antigens bound to the membrane by a Glicosil-Phosphatydil Inositol (MSA) have an important role in the bovine red blood cell invasion process by the parasite. In this report analyses on the polymorphism of Gp45, a B.bigemina GPI-anchored protein, are reported, providing the first reference to Italian sequences. Reported analyses allow to improve the knowledge on B.bigemina surface antigens, providing information about polymorphism and conservation of these antigens among geographically distant strains. (Financed by IZSSi02/07).

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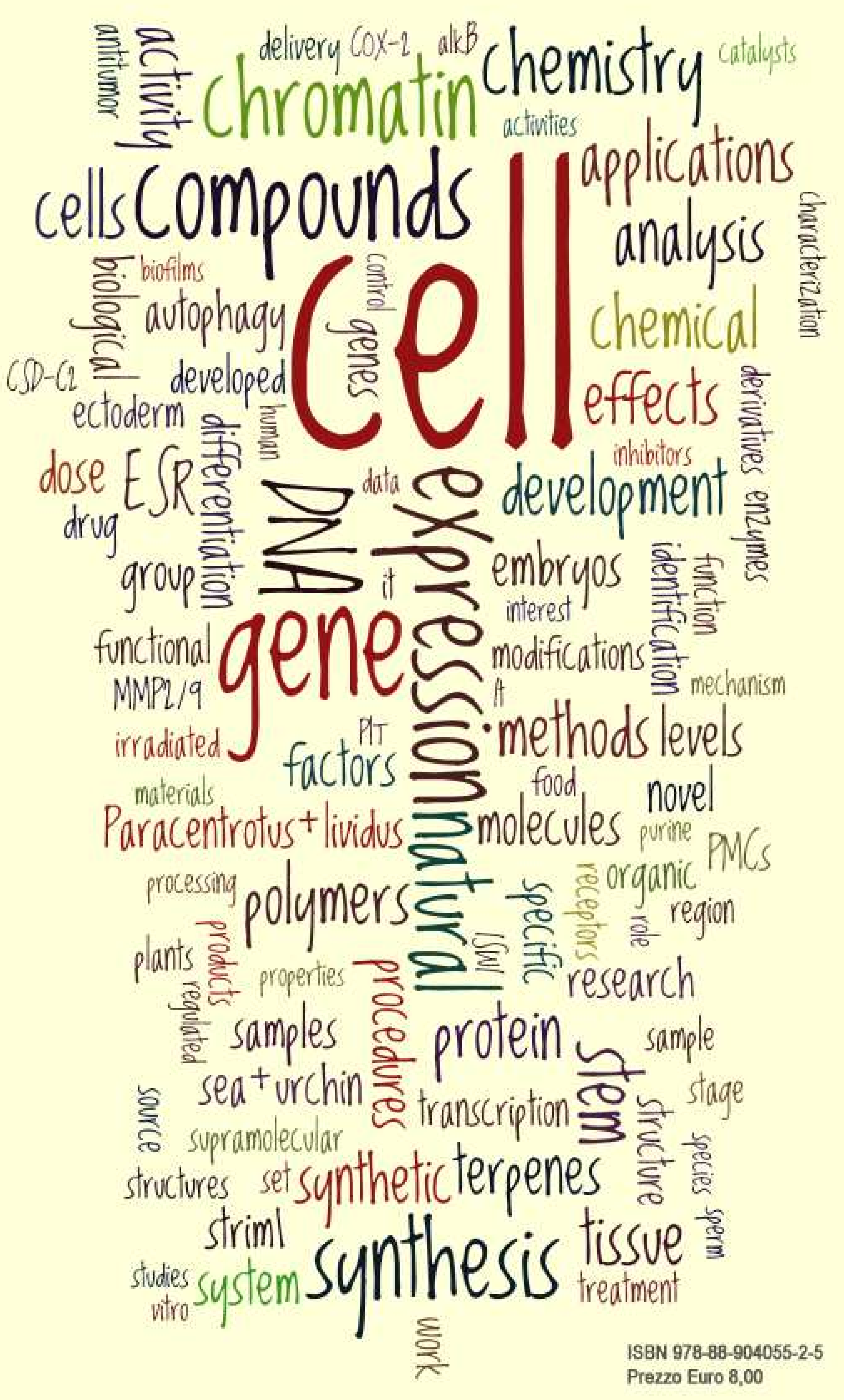

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