FROM BASIC RESEARCH TO TECHNOLOGY TRANSFER

IBPM Annual Meeting

Rome, May 3rd 2017
Aula Convegni CNR

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LA LUCE DELLA SCIENZA CERCO E TI BENEFICIO

SUMMARY

PROGRAMME

All titles of oral and poster presentations are hyperlinked to their abstracts

09.00 - 09.30 ***Opening***

Prof.ssa Clara Balsano, *Direttore Istituto di Biologia e Patologia Molecolari (IBPM)*

Prof. Giuseppe Novelli, *Rettore "Università degli Studi di Tor Vergata"*

Prof. Gennaro Ciliberto, *Direttore Scientifico IFO-IRE*

09.45-10.30 **Invited lecture: Dott. Giovanni Blandino**

Mutant p53 protein: an oncogenic regulator of coding and non-coding RNA network in human cancers

Oncogenomic and Epigenetic Unit, Regina Elena Cancer Institute, Rome

Session I: Cellular transformation and antitumoral therapy (IBPM)

10.30 - 10.45 **Annarita Favia,** Fiorella Scagnoli, Rosaria Anna Fontanella, Luisa Salvatori, Sergio Nasi, Barbara Illi
The Myc oncogene is differentially methylated by the Protein Arginine Methyltransferases 1 and 5 in glioblastoma stem cells

10.45 - 11.00 **Barbara Barbaro,** Cristiana Porcu, Laura Antonucci, Barbara Illi, Sergio Nasi, Clara Balsano
Biometals and modulation of MYC gene in the onset and progression of Hepatocellular Carcinoma

11.00-11.15 Manuela Leo, Elisa Cocco, Serena De Vito, Giulia Fanelli, Antonella Stoppacciaro, **Patrizia Filetici**
Epigenetic regulation and antitumoral effects

11.15 -11.45 ***Coffee break***

11.45 - 12.00 **Monica Carabelli,** Giovanna Sessa, Marco Possenti, Valentino Ruzza, Giorgio Morelli, Ida Ruberti
Arabidopsis HD-Zip II transcription factors regulate the exit from proliferation during leaf development in canopy shade

Session II: Pathogenesis of neurodegenerative diseases and new therapies for neuromuscular disease (IBPM)

12.00 - 12.15 Stefano Sechi, Anna Frappaolo, Roberta Fraschini, Giorgio Belloni,

Angela Karimpour Ghahnavieh, Roberto Piergentili, Michael Tiemeyer, **Maria Grazia Giansanti**
The role of membrane trafficking in cytokinesis and human diseases

12.15 - 12.30 P Laneve, L Piacentini, AM Casale, D Capauto, U Cappucci, P Di Micco, V Morea, CA Di Franco, **Elisa Caffarelli**
DendoU, a novel endoribonuclease linked to TDP-43-mediated neurodegeneration in D. melanogaster

12.30 - 12.45 **Gianluca Cestra**
P32:a mitochondrial protein that affects FUS- mediated ALS

12.45– 13.00 Loredana Biancolillo, Alessandra Pisciottani, Manuela Ferrara, Davide Valente, Francesca Sardina, Laura Monteonofrio, Serena Camerini, Marco Crescenzi, Silvia Soddu, **Cinzia Rinaldo**
The kinase HIPK2 regulates the microtubule severing factor spastin: role in cytokinesis and implications in neurological diseases

13.00-13.15 **Cinzia Pisani,** Maria Grazia Di Certo, Georgios Strimpakos, Annalisa Onori, Irene Carrozzo, Siro Luvisetto, Cinzia Severini, Francesca Gabanella, Stefano Farioli-Vecchioli, Lucia Monaco, Elisabetta Mattei, Nicoletta Corbi, Claudio Passananti
Artificial genes as therapeutic strategy for Duchenne Muscular Dystrophy (DMD)

13.15-14.30 ***Lunch and Poster session***

14.30-15.00 **Mario Incarnato**
Order Management System Software Project
AULA POLIFUNZIONALE

Session III: New perspectives in infectious diseases (IBPM)

15.00- 15.15 João B. Vicente, Henrique G. Colaço, Francesca Malagrinò, Paulo E. Santo, André Gutierres, Tiago M. Bandeiras, Paula Leandro, José A. Brito, **Alessandro Giuffrè**
The human hydrogen sulfide metabolism: Novel molecular mechanisms of pathogenicity

15.15 - 15.30 Rocchio S, Chiarella S, Franceschini M, Imperi F, Federici L, Travaglini-Allocatelli C, **Adele Di Matteo**
Tackling ribosome maturation process as antibacterial strategy: structural and functional investigation of RsgA from P. aeruginosa

Session IV: Structural protein studies for treatment of diseases (IBPM)

15.30- 15.45 **Gianni Colotti, Veronica Morea**
Stabilization of tRNA molecules as a therapeutic strategy for diseases due to mutations in mt-tRNAs

15.45 - 16.00 Giacomo Parisi, Linda Celeste Montemiglio, Antonella Scaglione, Beatrice Vallone, **Carmelinda Savino**
Structural Plasticity in Macrolide Biosynthetic Cytochrome P450s

Prof.ssa Clara Balsano *Remarks of the IBPM Director*

Posters

Giuliana Righi, Paolo Bovicelli, Alessandra Ricelli, Carla Sappino, Lorenza Suber
Preparation of nanostructured chiral catalysts

Paolo Bovicelli, Giuliana Righi, Fabrizio Bottaro, Carla Sappino, Caterina Frezza, Beatrice Macchi
Valorization of waste: from tyrosol to biologically active polyphenols

Giuliana Righi, Paolo Bovicelli, Carla Sappino
Stereocontrolled synthesis of azasugar D-1-deoxygalattonojrimycin

Giovanna Costanzo, Samanta Pino, Ernesto Di Mauro, Raffaele Saladino, Jiri Sponer, Judit Sponer
From formamide to RNA the prebiotic path is tenuous but continuous

Angela Tramonti, Roberto Contestabile, Martino L. di Salvo, Stefano Pascarella
Regulation of Vitamin B6 metabolism in bacteria: involvement of the MocR family of pyridoxal phosphate-dependent transcriptional regulators

Annalisa Onori, Cinzia Pisani, Maria Grazia Di Certo, Georgios Strimpakos, Francesca Gabanella, Francesca Delle Monache, Irene Carrozzo, Lucia Monaco, Elisabetta Mattei, Yaffa Beck, Nicoletta Corbi, Claudio Passananti
Merosin-deficient Congenital Muscular Dystrophy type 1A (MDC1A): an innovative therapeutic strategy based on Zinc Finger Artificial Transcription Factors (ZF-ATFs)

Paola Fragapane, Francesca Cosmi, Irene Bozzoni, Maria Egle De Stefano
Hippocampal neurons of dystrophic *mdx* mice are less responsive than wild type to acute corticosterone treatment *in vitro*

Micaela Caserta, Sabrina Venditti, Valerio Vetriani,Tal Dotan Ben-Soussan, Loredana Verdone
Neurotrophin level changes induced by QMT, a new cognitive-motor training

Cecilia Mannironi, Silvia Gasparini, Valerio Licursi, Giorgia Del Vecchio, Ivano Legnini, Arianna Rinaldi, Anna Maria Scattoni, Carlo Presutti
Circular RNAs in Autism Spectrum Disorder

Fabio Di Domenico, Antonella Tramutola, Nidhi Sharma, Cesira Foppoli, Tommaso Cassano, Marzia Perluigi
Altered protein *O*-GlcNAcylation profile revealed by proteomics: novel insights on protein signalling mechanisms in Alzheimer Disease

Eugenia Gilistro, Lia Asteriti, Annalisa Verrico, Laura Di Francesco, Paola Rovella, Pietro Cirigliano, Eugenia Schininà, Giulia Guarguaglini, Patrizia Lavia
In situ detection of protein interactions and modifications by proximity-ligation assay

Jessica Bartoli, Erica Di Cesare, Sara Moroni, Michela Damizia, Patrizia Lavia
SUMO-conjugation of the Aurora-B kinase is essential during mitosis

Claudia Pellacani, Elisabetta Bucciarelli, Fioranna Renda, Daniel Hayward, Antonella Palena, Jack Chen, Silvia Bonaccorsi, James G. Wakefield, Maurizio Gatti, Maria Patrizia Somma

The Sf3A2 and Prp31 splicing factors play a direct role in mitotic chromosome segregation

Roberto Antonioletti, Giuliana Righi, Alessandra Ricelli, Ilaria Rossetti, Angela Viglianti, Caterina Frezza, Francesca Marino-Merlo, Beatrice Macchi

Synthesis and biological evaluation as apoptosis-inducing agents of styrylheterocycles analogs of resveratrol

Elisabetta Falvo, Pierpaolo Ceci, Luciana Mosca, Elena Poser, Ilaria Genovese, Giulia Guarguaglini, Gianni Colotti

Use of Ferritin-Based Metal-Encapsulated Nanocarriers as Anticancer Agents

Manuela Ferrara, Gaetana Sessa, Mario Fiore, Francesca Bernard, Italia Anna Asteriti, Enrico Cundari, Gianni Colotti, Salvatore Ferla, Marianna Desideri, Donatella Del Bufalo, Andrea Brancale, Francesca Degrassi

Small molecules targeted to the microtubule-Hec1 interaction inhibit cancer cell growth through microtubule stabilization

Marta Di Martile, Simonetta Buglioni, Nicola Baldini, Roberto Biagini, Donatella Del Bufalo, Daniela Trisciuoglio

Chemosentization of sarcoma cells by ITF2357 histone deacetylase inhibitor

Luisa Salvatori, Rosaria Anna Fontanella, Annarita Favia, Barbara Illi

Nitric oxide markedly affects glioblastoma stem cells' phenotype and differentiation capacity

Gianna Fiorucci, Marco Iuliano, Giorgio Mangino, Maria Vincenza Chiantore, Giovanna Romeo

Inflammatory microenvironment and human papillomavirus-induced carcinogenesis

Sonia Simonetti, Amairelys Belen Barroeta Seijas, Sara Vitale, Daniele Runci, Angela Caterina Quinci, Alessandra Soriani, Mattia Criscuoli, Irene Filippi, Antonella Naldini, Federico Maria Sacchetti, Umberto Tarantino, Francesco Oliva, Eleonora Piccirilli, Angela Santoni, Francesca Di Rosa

GM-CSF inhibits c-kit and SCF expression by Dendritic Cells

Mario Incarnato

Order Management System Software Project

ORAL PRESENTATIONS

O-1: The Myc oncoprotein is differentially methylated by the Protein Arginine Methyltransferases 1 and 5 in glioblastoma stem cells

Annarita Favia[1], Fiorella Scagnoli[1,2], Rosaria Anna Fontanella[1], Luisa Salvatori[1], Sergio Nasi[1], Barbara Illi[1]

[1] Institute of Molecular Biology and Pathology – National Research Council (IBPM-CNR), Rome, Italy
[2] Department of Biology and Biotechnology, Sapienza University of Rome, Rome, Italy

Background. The Myc oncogene governs RNA polymerase II (RNA PolII) pause release, transcriptional elongation and chromatin structure by triggering histone modifications. Type I and II Protein Arginine (R) Methylatransferases (PRMTs) catalyze methylation of many cytoplasmic and nuclear substrates on R residues. PRMT1, belonging to type I, catalyze mono- and asymmetric dimethylations. The type II PRMT5 catalyze mono- or symmetric dimethylation exerting its catalytic activity through the association with a co-factors, such as the Methylosome Protein 50 (MEP50). Both proteins regulates cell signaling, chromatin remodeling and gene expression and have been found dysregulated in many cancers. Further, both PRMT5 and PRMT1 are able to di-methylate the neuronal isoform of Myc (N-Myc). Glioblastoma multiforme (GBM) is a rare, invariably lethal brain tumor, and valuable therapeutic strategies are urgently needed.

Results. Our data show that, in HEK293T cells transfected with a plasmid overexpressing a Flag– tagged Myc, FlagMyc overexpression induces both symmetric and asymmetric di-methylation of R 3 on histone H4 (H4R3me2s), as revealed by western blot. Consistently, in these cells, FlagMyc associates with both PRMT5 and PRMT1. The same result was obtained in glioblastoma stem cells (GSCs). Therefore, we asked whether Myc was methylated. In reciprocal immunoprecipitation experiments, in FlagMyc expressing HEK293T cells, we have found that antibodies raised against asymmetric and symmetric di-methylated R, recognized the FlagMyc protein. Conversely, in GSCs, grown in stemness conditions, symmetrically di-methylated Myc was the main di-methylated Myc isoform. On the contrary, in differentiating GSCs, a shift to Myc asymmetric di-methylation was observed. To unveil the functional significance of these di-methylations, we investigated Myc protein stability upon GSCs exposure to PRMT5 and PRMT1 inhibitors, followed by cycloheximide (CHX) and MG132 treatment. We found that, in FlagMyc HEK293T expressing cells, exposure to the PRMT5 inhibitor had, basically, no effect on FlagMyc stability; the PRMT1 inhibitor MC2010, however, enhanced Myc stability upon CHX exposure. Blocking the proteasome pathway, by MG132 administration, led to an increase in Myc stability, as expected, both in the presence of EPZ01566 and MC2010. In GSCs, we found that PRMT5 inhibition had a dramatic detrimental effect on Myc stability, upon CHX exposure. PRMT1 inhibition, also, had a negative effect on Myc protein stability, but to a lesser extent. MG132 treatment, restored Myc protein levels in EPZ01566 treated cells; interestingly, in the presence of

the PRMT1 inhibitor, Myc protein levels increased about 5 fold, when compared to solvent treated cells, upon MG132 treatment.

Conclusions. Our data show that Myc is di-methylated by both PRMT5 and PRMT1. PRMT5 seems to protect endogenous Myc from degradation, while PRMT1, probably, has the opposite effect. This is consistent with the oncogenic role of both Myc and PRMT5, which are highly overexpressed in GBM and GSCs, and with the prevalence of asymmetrically di-methylated Myc in differentiating GSCs. We suggest that these Myc post-translational modifications may be considered as novel therapeutic targets for GBM treatment.

O-2: Biometals and modulation of Myc gene in the onset and progression of Hepatocellular Carcinoma

Barbara Barbaro[1], Cristiana Porcu[2], Laura Antonucci[2], Barbara Illi[1], Sergio Nasi[1], Clara Balsano[1]

[1] Institute of Molecular Biology and Pathology (IBPM) - CNR, Rome, Italy
[2] Laboratory of Molecular Virology and Oncology, Francesco Balsano Foundation, Rome, Italy

Background. Hepatocellular carcinoma (HCC) represents the third most frequent cause of cancer death. Biometals metabolism results significantly altered in tumors: in particular, high serum and tissue levels of copper were found in HCC patients. Myc deregulation and overexpression represent major mechanisms of tumorigenesis in a variety of cancers, including HCC.
Aim. The purpose of this study was to evaluate the role of copper in hepatic tumorigenesis, deepening on the interplay with Myc. To this aim, the effects of copper treatment on normal hepatic cells were assessed, alongside with the effects of a copper chelator (Ammonium-tetratiomolibdate, TTM) or a dominant negative protein that counteracts Myc activity (Omomyc), in HCC cell lines.
Materials and Methods. Human hepatic HepaRG cells were treated with different concentrations (20, 35 and 50μM) of CuSO4, while human hepatoma HepG2 and HuH7.5 cells were treated with 50 and 100 μM of TTM or engineered to express Omomyc under the control of a doxicycline inducible promoter. We evaluated the intracellular copper content by atomic absorption spectroscopy, cell viability by MTS assay, gene expression by RT-PCR, protein levels by western blot, the binding of Myc on Ctr1 promoter by ChIP. Cell cycle and death were analyzed by FACS and migration and invasivity with scratch and invasion assays.
Results. HepaRG cells treated with CuSO4 incorporated copper and showed an increase of S and G2/M phases, associated with an elevated expression of Pcna and cyclins, with no increase of cell death. We highlighted that copper induced a significant up-regulation of Myc and an increased binding of Myc on Ctr1 promoter, the main transporter of copper in hepatocytes. Copper induced also the migration and matrix invasion of HepaRG that correlated with the down-regulation of E-cadherin and the up-regulation of β-catenin mRNA and protein levels. The activation of Omomyc or the treatment with TTM in HepaRG reversed all the results. On the other hand, HepG2 and HuH7.5 showed high basal level of copper: the activation of Omomyc, as well as the treatment with TTM, determined a reduction of the intracellular copper content and a reduced expression of Ctr1, Pcna and Myc with a consequent reduction of proliferation, migration and invasion.
Conclusions. Our data indicate that in hepatocytes exists a positive feedback between copper intake and Myc that might push towards transformation. Thus, high levels of copper in liver parenchyma could contribute to the onset of a favorable condition for cell transformation and, on the other hand, therapies that accounts a reduction of Myc activity and a copper chelation should be considered to counteract HCC progression.

O-3: Epigenetic regulation and antitumoral effects

Manuela Leo, Elisa Cocco, Serena De Vito, Giulia Fanelli, Antonella Stoppacciaro, Patrizia Filetici

Institute of Molecular Biology and Pathology (IBPM) - CNR, Rome, Italy

Based on the results obtained in our group in the past, we have approached a novel study on the role exerted by K-acetyltransferases and novel inhibitory compounds on tumor cell lines. We base our experimental strategy on findings obtained in the simple yeast model system. Kidney clear cell carcinoma ccRCC is responsible for the majority of primary renal neoplasia, it is substantially insensible to current therapies and the efficacy of treatment is directly related to the dissemination of the tumor. In our study, we evaluated the pharmacological effects of K-histone acetyltransferase inhibitor CPTH2 and showed its preferential inhibition for p300 with ipoacetylating effects on histone H3 and at specific H3AcK18. Tumor tissues were compared with peritumoral normal epithelium in 70 ccRCC case patients. Collected results indicate that CPTH2 is a novel compound counteracting progression of asymptomatic and risky ccRCC.

O-4: Arabidopsis HD-ZIP II transcription factors regulate the exit from proliferation during leaf development in canopy shade

Monica Carabelli[1], Giovanna Sessa[1], Marco Possenti[2], Valentino Ruzza[1], Giorgio Morelli[2], Ida Ruberti[1]

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In the last decades, an increasing body of evidence highlights the significance of the reduction in the Red(R)/Far-Red(FR) ratio of light as a signal that triggers shade avoidance response. This response is mainly appreciable as increased plant elongation at the expenses of leaf and root expansion. Despite the significant advances in understanding the mechanisms underlying shade-induced hypocotyl elongation (Casal, 2013), little is known about the cellular and molecular responses to Low R/FR in organs other than hypocotyl. In Arabidopsis, there is evidence that cell number contributes to the reduced leaf size of plants grown under simulated shade. Low R/FR rapidly and transiently reduces the frequency of cell division in young leaf primordia through a non-cell autonomous mechanism that requires the action of the auxin receptor TRANSPORT INHIBITOR RESISTANT 1 (TIR1). The auxin increase perceived through TIR1 induces CYTOKININ OXIDASE/DEHYDROGENASE 6 (CKX6), a gene encoding an enzyme involved in cytokinin degradation, which in turn promoting cytokinin breakdown diminishes cell proliferation in developing leaf primordia. Interestingly, the up-regulation of DR5::GUS as well as that of CKX6::GUS by low R/FR light occurs in pre-provascular cells of young leaf primordia, suggesting that induction of cytokinin degradation in the developing vasculature may be sufficient to transiently arrest leaf primordium growth in Low R/FR (Carabelli et al. 2007, 2008). However, it remains unknown whether the rapid and transient arrest in growth of the leaf primordia is the only effect of shade on Arabidopsis leaf development. Here, we first show that prolonged Low R/FR determines early exit of proliferation in the leaf and secondly, through the analysis of single and multiple HD-Zip II-γ subfamily mutants, that this requires the HD-ZIP II transcription factors ATHB2 and ATHB4.

References:

1. Casal JJ. Annu Rev Plant Biol. 2013;64:403-27.
2. Carabelli M, Possenti M, Sessa G, Ciolfi A, Sassi M, Morelli G, Ruberti I. Genes Dev. 2007 Aug 1;21(15):1863-8.
3. Carabelli M, Possenti M, Sessa G, Ciolfi A, Sassi M, Morelli G, Ruberti I. Plant Signal Behav. 2008 Feb;3(2):137-9.

O-5: The role of membrane trafficking in cytokinesis and human diseases

Stefano Sechi[1], Anna Frappaolo[1], Roberta Fraschini[2], Giorgio Belloni[1], Angela Karimpour Ghahnavieh[1], Roberto Piergentili[1], Michael Tiemeyer[3], Maria Grazia Giansanti[1]

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In animal cell cytokinesis, constriction of a plasma membrane-anchored actomyosin ring leads to cleavage furrow ingression at the equatorial cortex. Recent data indicate the requirement for membrane trafficking during cleavage furrow ingression as well as during abscission.
The Conserved Oligomeric Golgi (COG) complex mediates tethering of vesicles carrying glycosylation enzymes across the Golgi cisternae. Mutations in human *COG7* and other *COG* genes cause distinct forms of inherited, autosomal recessive, congenital disorders of glycosylation (CDG) associated with multisystemic deficiencies. Patients suffering from COG7-CDG typically exhibit microcephaly, epileptic seizures, and brain atrophy. Yet, the correlation between defective glycosylation and the neurological aspects of the pathology remains unknown. Given the complexity of glycan composition and the redundancy of glycosylation enzymes in vertebrate cells, *Drosophila* offers unique opportunities for neural glycomics studies with the ease of genetic tools and well-established neurobiological approaches.
We have recently shown that *Drosophila* Cog7 is required for Golgi trafficking and normal cytokinesis in both spermatocytes and larval neuroblasts. Remarkably cytokinesis failures have been proposed to affect neurogenesis thus contributing to microcephaly, one of the neurological trait of COG7-CDG patients. We are dissecting the molecular interactions involving Cog7 other vesicle trafficking proteins involved in brain cytokinesis. In addition, we are collaborating with the Tiemeyer laboratory for profiling glycoproteins and glycolipids from fly heads of wild type and *Cog7* mutants by the use of multi-dimensional ion trap mass spectrometry. Overall our analysis should shed light on the molecular circuits involving Cog7 in brain cytokinesis. Moreover our studies should elucidate the connection between altered glycosylation and the neuropathology of COG7-CDG and help identify novel therapeutic strategies. Moreover our CDG *Drosophila* model might also serve as a platform to investigate other COG-CDGs.

O-6: DendoU, a novel endoribonuclease linked to TDP-43-mediated neurodegeneration in *D. melanogaster*

P. Laneve[1], L. Piacentini[2], A. M. Casale[2], D. Capauto[1], U. Cappucci[2], P. Di Micco[3], V. Morea[3], C. A. Di Franco[2], E. Caffarelli[3]

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Endoribonucleases are central players in various pathways of eukaryotic RNA metabolism. We previously identified the founding member of the Eukaryotic EndoU ribonuclease family, whose components are flexibly involved in important biological processes, such as ribosome biogenesis, tumorigenesis and viral replication. By combining bioinformatics and biochemical analyses we identified a novel member of this family, that we named DendoU, in *D. melanogaster*. Exploiting the powerful reverse-genetics tools of the fruit fly model, we investigated DendoU in vivo activity. The protein was found to be essential for Drosophila viability and crucial for neuronal activity. Pan-neuronal silencing of DendoU resulted in fly immature phenotypes, highly reduced lifespan and dramatic motor performance defects. These phenotypes overlap with those caused by loss- and gain-of-function of the neurodegeneration-associated protein dTDP-43, which we revealed to be regulated by DendoU.

Figure 1: ELAV>CG3303^{RNAi} adult escapers display a juvenile phenotype

O-7: P32: a mitochondrial protein that affects FUS-mediated ALS

Gianluca Cestra

Institute of Molecular Biology and Pathology, National Research Council, Rome

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disorder caused by motor neuron loss. Mutations in the RNA binding protein FUS are associated to familiar forms of the disease. An *in vivo Drosophila* model of FUS-mediated neurodegeneration has been established: FUS mutations reduce fly viability and alter its locomotion activity. In an affinity purification experiment, we have identified P32 as one of the proteins that specifically bind to FUS. P32 is expressed in motor neurons and has several mitochondria-related properties. RNAi-mediated downregulation of P32 in *Drosophila* reduces fly locomotion. More interestingly, upregulation of P32 expression in flies carrying FUS mutations partially suppress FUS-mediated neurodegeneration suggesting that alterations of P32 activity may be relevant for the pathogenesis of ALS due to FUS mutation.

O-8: The kinase HIPK2 regulates the microtubule severing factor spastin: role in cytokinesis and implications in neurological diseases

Loredana Biancolillo[1], Alessandra Pisciottani[1], Manuela Ferrara[1], Davide Valente[1,2], Francesca Sardina[1], Laura Monteonofrio[2], Serena Camerini[3], Marco Crescenzi[3], Silvia Soddu[2], Cinzia Rinaldo[1,2]

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[3] Istituto Superiore di Sanità ,Roma

The oncosuppressor HIPK2 is a kinase controlling DNA damage-induced cell fate and cytokinesis [1-2-3]. During studies on the molecular basis of HIPK2 role in cytokinesis and in ploidy maintenance, we found that HIPK2 directly regulates the protein levels of the microtubule severing protein spastin, whose activity is essential for the abscission. HIPK2 depletion leads to spastin downregulation in a proteasome-dependent manner. Wild-type HIPK2 overexpression, but not its kinase-dead mutant, increases spastin protein levels, suggesting that kinase activity is required for spastin regulation. Furthermore, we found that HIPK2 specifically phosphorylates spastin. We have identified the spastin serine residue phosphorylated by HIPK2 and we are generating non phosphorilatable and phoshomimetic mutants.
Furthermore, we will present implications of the HIPK2/spastin cross talk in Hereditary Spastic Paraplegia (HSP), a neurodegenerative disorder mainly due to heterozygous mutations in the SPG4 gene, encoding spastin. We are investigating the molecular mechanism/s underlying HIPK2 mediated spastin regulation in motoneurons. Preliminary experiments will be also performed on neurons from HSP SPG4 patients.

References:

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2. Rinaldo C, Moncada A, Gradi A, Ciuffini L, D'Eliseo D, Siepi F, Prodosmo A,Giorgi A, Pierantoni GM, Trapasso F, Guarguaglini G, Bartolazzi A, Cundari E, Schininà ME, Fusco A, Soddu S. HIPK2 controls cytokinesis and prevents tetraploidization by phosphorylating histone H2B at the midbody. Mol Cell, 2012, 47:87-98
3. Valente D, Bossi G, Moncada A, Tornincasa M, Indelicato S, Piscuoglio S, Karamitopoulou ED, Bartolazzi A, Pierantoni GM, Fusco A, Soddu S, Rinaldo C. HIPK2 deficiency causes chromosomal instability by cytokinesis failure and increases tumorigenicity. Oncotarget, 2015, 6:10320-10334

O-9: Artificial genes as therapeutic strategy for Duchenne Muscular Dystrophy (DMD)

Cinzia Pisani[1] , Maria Grazia Di Certo[2], Georgios Strimpakos[2], Annalisa Onori[1], Irene Carrozzo[1], Siro Luvisetto[2], Cinzia Severini[2], Francesca Gabanella[2], Stefano Farioli-Vecchioli[2], Lucia Monaco[3], Elisabetta Mattei[2], Nicoletta Corbi[1], Claudio Passananti[1]

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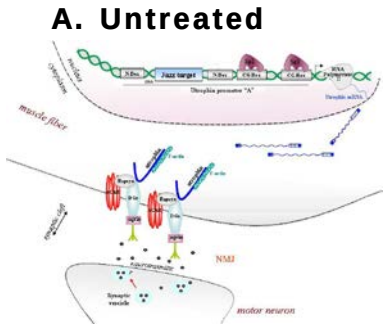

Figure 1. The artificial transcription factor JZif1 (and its prototype Jazz) impacts the neuromuscular junction (NMJ) by utrophin up-regulation. a. The panel illustrates a schematic representation of the utrophin-associated protein complex at mammalian neuromuscular junction (NMJ). Dystroglycan (DG) associates with rapsyn and rapsyn links Acetylcholine Receptors (AChRs) to utrophin. Utrophin links the entire complex to the F-actin cytoskeleton. b. The artificial transcription factor JZif1 binds the utrophin promoter "A" at the 9 base pair long target sequence, thereby enhancing transcription of utrophin gene. The expression of JZif1 (and Jazz) gene improves the morphology/remodelling of NMJ.

Up-regulation of the dystrophin-related gene "utrophin" represents a promising therapeutic strategy for the treatment of Duchenne Muscular Dystrophy (DMD). In order to re-program the utrophin expression level in muscle, we engineered artificial zinc finger transcription factors (ZF-ATFs) that target the utrophin promoter ''A''. In dystrophic "mdx" mice by systemic Adeno-associated viral vector delivery, the prototype artificial gene "Jazz" or its up-graded version "JZif1" induces significant muscle functional rescue. These encouraging results are producing several international patents owned by CNR and financially supported by Zingenix Ltd Company (Tel Aviv, Israel). To investigate the molecular mechanisms underlying Jazz and JZif1 induced muscle rescue, we focused on utrophin related pathways. In particular, on-going studies aim to characterize the neuromuscular junction (NMJ) in Jazz and JZif1 mAAV8-treated wt and mdx mice. We have analyzed, in skeletal muscle, pre- and post-synaptic structures by staining the neurofilaments and acetylcholine receptor (AChR) clusters, respectively. Based on preliminary results, our ZF-ATF genes provide a potential benefit on NMJ morphology, improving the post-synaptic clustering of AChRs. In summary, the development of ZF-ATF technology, coupled with the mAAV delivery, highlights the potential of this novel therapeutic strategy for DMD pre-clinical animal model.

O-10: Order Management System Software Project

Mario Incarnato

CNR–National Research Council of Italy, Institute of Molecular Biology and Pathology, Rome, Italy

The software application aims to provide support for the drafting and management of purchase orders for equipment and services useful to research and to institute administrative activities. The application simplifies the administrative process flow related to orders, from their initiation to the various intermediate stages, until completion and sending to the supplier.
Gathering all necessary requirements led to the definition of the main subjects using the application, being the Compiler, the Funds Manager, the Administrative Representative and the Director.
The order application process typically begins by filling out a decision to be negotiated, so the application provides an interactive guide function, based on the default standard MS WORD template of the institute, where the Compiler inserts the required data; a PDF document is then produced, validated and automatically available for the next stage, the completion of the declaration of the RUP. Also in this case, the application facilitates the compilation and production of the resulting PDF document, carried out by the Funds Manager.
The requested order is completed by the Compiler, who through another guided interactive function, also based on the institute's standard MS WORD template, inserting order data and producing another PDF document.
The workflow is controlled by the Administrative Representative, who may reject the submitted documents, providing reasons, for example requesting correction of details, or validate and accept the documents.
The next step is determined by the final validation of the Director, after which the documents can be forwarded to computer protocol and the order can be sent to the supplier.
The application is created with open source technologies and frameworks, including Java 8, Spring Framework, AngularJS 1.5.8, Bootstrap 3.3.7 and 2.4 Elasticsearch, combined together by JHipster generator 3.12.2.

O-11: The human hydrogen sulfide metabolism: novel molecular mechanisms of pathogenicity

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Hydrogen sulfide (H_2S), currently recognized as the third 'gasotransmitter' in addition to nitric oxide (NO) and carbon monoxide (CO), plays a key signalling role in human physiology and pathophysiology. A growing number of pathologies, including several metabolic, cardiovascular, oncologic and neurodegenerative diseases, are indeed reportedly associated with alterations of H_2S metabolism. Among metabolic diseases, classical homocystinuria results from mutations in the gene encoding the pyridoxal 5'-phosphate (PLP)-dependent cystathionine β-synthase (CBS), a key enzyme that controls homocysteine levels and is a major source of H_2S in humans. CBS, contributing to cellular redox homeostasis, is positively regulated by s-adenosyl-l-methionine (AdoMet), but fully inhibited upon CO or NO• binding to a non-catalytic heme moiety [1,2], being thus implicated in the interplay between gasotransmitters. Despite extensive studies, the molecular basis of classical homocystinuria is not yet fully understood. We have recently reported [3] that the ferrous heme of the reportedly mild p.P49L CBS variant has altered spectral properties and markedly increased affinity for CO, making the protein much more prone than wild type CBS to inactivation at physiological CO levels. The higher CO affinity could result from the slightly higher flexibility in the heme surroundings revealed by solving the crystallographic structure of a truncated p.P49L at 2.80-Å resolution. Additionally, p.P49L CBS displays impaired H_2S-generating activity that is rescued by PLP supplementation along the purification. We suggest that the higher propensity to CO inactivation documented here for p.P49L CBS could represent a novel pathogenic mechanism in classical homocystinuria.

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O-12: Tackling ribosome maturation process as antibacterial strategy: structural and functional investigation of RsgA from *P. aeruginosa.*

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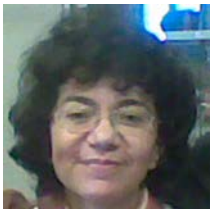
The opportunistic bacterium *Pseudomonas aeruginosa* causes serious infections in immunocompromised and hospitalized patients. Moreover, it is the major pathogen in the cystic fibrosis. One of the most worrisome characteristics of *P. aeruginosa* is its low antibiotic susceptibility and its ability to develop acquired resistance.

We have characterized from a structural and functional point of view RsgA (Ribosome small subunit-dependent GTPase) from this pathogen (*Pa*RsgA). Rsga, a circularly permutated GTPase (cGTPase), is a late-stage ribosome biogenesis factor involved in the 30S subunit maturation. It belongs to the TRAFAC GTPases and shares the common characteristic of a circularly permutated GTP binding site in which the classical G domain motif are arranged as G4-G5-G1-G2-G3 in comparison to the classical G-motif arrangement (G1-G2-G3-G4-G5). In RsgA the central GTPase motif is flanked by an OB-fold (oligonucleotide/ oligosaccharides binding fold) domain at the N-terminus and by a Zn knuckle-like cysteines region at the C-terminus. Both OB-fold and Zn motif have been found in various RNA-binding proteins.

We solved the 3D structure of *Pa*RsgA in complex with GDP at 2.8 Å resolution and we carried out detailed kinetic and equilibrium analysis of the GDP/GTP binding to *Pa*RsgA using MANT-guanine nucleotides analogues as well as a characterization of its GTPase activity. Moreover, in vivo genetic experiments show that *RsgA* is important for *P. aeruginosa* virulence against *Galleria mellonella* moth. These results, strengthening the hypothesis of its role as checkpoint protein in ribosome biogenesis process, pose RsgA as a potential drug target for *Pseudomonas* infection treatment.

O-13: Stabilization of tRNA molecules as a therapeutic strategy for diseases due to mutations in mt-tRNAs.



Veronica Morea , Gianni Colotti

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Background. Mitochondrial (mt) diseases are multi-system disorders due to mutations in nuclear or mtDNA genes. Among the latter mutations, more than 50% are located in transfer RNA genes and are responsible for a wide range of currently untreatable pathologies.

Rationale. Defects due to point mutations in several mt-tRNAs are rescued by overexpression of the cognate human mt-aaRS in both the yeast model and human cells. In 3D structures, tRNALeu interacts extensively with the carboxy-terminal domain of LeuRS (LeuRS-Cterm, comprising ~60 amino acid residues), and in particular with residues within β-strands β30, β31, β32 and β33.

In the yeast model, both the isolated human mtLeuRS-Cterm and peptides β30-31 (15 residues) and β32-33 (16 residues), comprising two β-strands each, rescue defects due to point mutations in several mt-tRNAs aminoacylated by aaRSs belonging to either class I or class II.

Results. LeuRS-Cterm: i) is the LeuRS region required and sufficient to rescue the pathological phenotype of human cells (cybrids) carrying point mutations associated with either severe pathologies (*i.e.*, m.4234A>G in mt-tRNALeu(UUR), causing MELAS; and m.8344A>G point mutation in mt-tRNALys, causing MERRF) or relatively mild syndromes (*i.e.*, m.4277T>C and m.4300A>G in mt-tRNAIle, causing cardiomyopathies); ii) localizes to mitochondria even in the absence of a canonical N-terminal mt targeting sequence; iii) directly and specifically interacts *in vitro* with cognate human mt-tRNALeu(UUR) with high affinity, and with non-cognate human mt-tRNAIle with lower affinity.

Short peptides β30-31 and β32-33: i) ameliorate viability and energetic proficiency of human cells carrying both of the aforementioned point mutations causing severe pathologies; ii) interact *in vitro* with cognate human mt-tRNALeu(UUR) with high affinity, with the m.3243A>G mutant with lower affinity, and with non-cognate wt and mutant mt-tRNALys, with lower affinity than cognate tRNAs; iii) restore the structure and thermal stability of mt-tRNALeu(UUR), which are strongly impaired by the m.3243A>G mutation.

Conclusions and Perspectives. We have demonstrated that short peptides derived from LeuRS are able to rescue the pathological phenotype of the two most common and severe mt diseases caused by point mutations in mt-tRNA genes in human cells, for which no therapy is available at present. The ability of these peptides to directly interact with, and restore the conformation and stability of mt-tRNAs suggests that the rescuing activity is mediated by a “chaperonic” mechanism. The two peptides are therefore attractive lead molecules for the development of therapeutic compounds against mt-tRNA mutations-related syndromes.

To translate these results into therapeutic applications, we aim to:

1. develop strategies to deliver rescuing molecules to the mitochondria;
2. identify novel non-peptidic compounds endowed with rescuing activity by either rational design or screening of large collections of small organic molecules for their ability to stabilize a wild-type like conformation of mt-tRNA mutants.

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O-14: Structural Plasticity in Macrolide Biosynthetic P450s.

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Cytochrome P450s are heme-containing proteins that catalyze the oxidative metabolism of many endogenous compounds. One of the most peculiar aspects of their activity is that, depending on their physiological role, the same overall fold and a common catalytic mechanism are exploited to perform chemically different reactions on a plethora of alternative compounds. Such versatility is due to the flexibility and to the modularity of the secondary structural elements that constitute the scaffolding of the active site [1].
I will present the case of two bacterial P450s involved in macrolide antibiotic biosynthesis where the common fold has been adapted for binding and oxidation of substrates markedly different in size.
The C12 hydroxylase EryK from *Saccharopolyspora erythraea* is a P450 involved in erythromycin biosynthesis that catalyses the hydroxylation of the biosynthetic intermediate erythromycin D, a double-glycosylated 14-membered macrolide [2].
OleP, from *Streptomyces antibioticus*, is a P450 epoxygenase that acts on both the aglicone and on the mono-glycosylated intermediates of the antibiotic oleandomycin, the actual substrate still remaining unclear [3].
A crystallographic analysis on both EryK and OleP in conjunction with an extensive functional characterization of binding mechanism to their substrates and to P450 inhibitors revealed these enzymes to show different structural plasticity and alternative mechanisms of ligand binding, with a diversification of the structural elements that open and close the active site [4-7].

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POSTERS

Preparation of nanostructured chiral catalysts

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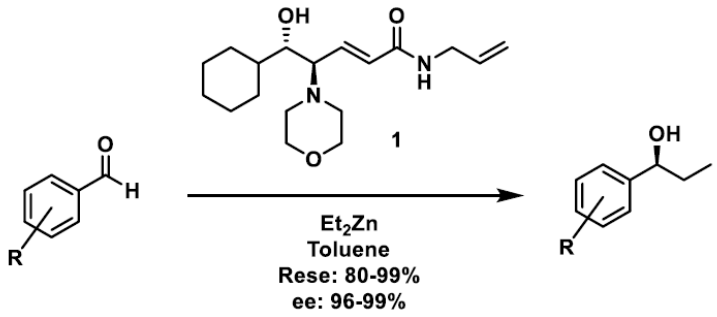
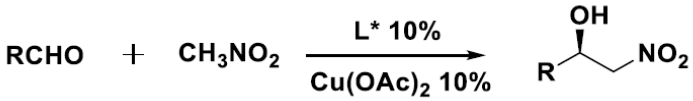
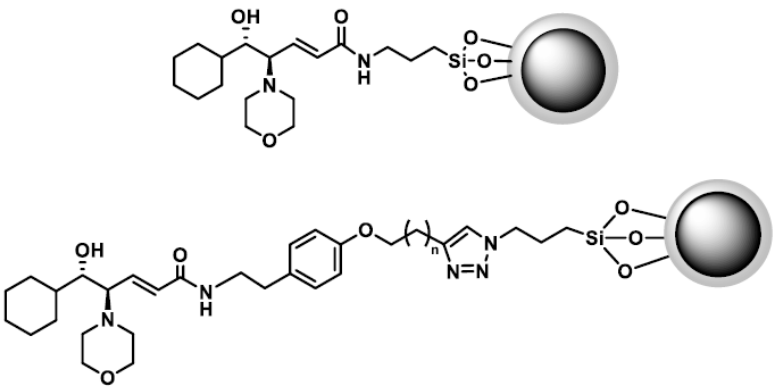
In the last years, the growing study of nanostructured materials has given the birth to a variety of new applications in many fields, among which catalysis. New chiral nanosystems have been developed, combining advantages of both homogeneous and heterogeneous catalysis nanoparticles' dispersibility in organic solvents makes their catalytic activity close to that of the homogeneous counterparts, and at the same time, they are easily separated from the reaction mixture leading to an economical and environmental benefit [1].
Considering the presence of the β-amino alcohol motif in the structure of numerous chiral catalysts used in asymmetric synthesis [2], we were recently involved in the development of a novel versatile, magnetically recoverable β-amino alcohol 'nanocatalyst'.
Before immobilizing the catalyst on the nanoparticles, we dealt with the design and the optimization of the ligand's structure, and after an extensive fine-tuning process, we selected **1** as an excellent chiral ligand in the addition of diethylzinc to aldehydes (yields: 88-99% *ee*: 96-99%).



We are now focusing on the optimization of the employment of ligand **1** in the asymmetric version of another important organic reaction, the nitroaldol or Henry reaction [3], source of nitroalcohols, a synthetically useful class of compounds.



Together, we dealt with the choice of the best nanosized support and with the optimization of the immobilization conditions. We selected silica and magnetite-silica core shell nanoparticles. The latter show a superparamagnetic behavior, allowing a quick recovery by magnetic decantation.
Regarding the immobilization step, we followed two different strategies that involved a condensation or a click chemistry reaction.
The optimized nanocatalyst will be tested in the same reactions studied in the homogeneous phase, the addition of diethylzinc and nitromethane to aldehydes.



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Valorization of waste: from tyrosol to biologically active polyphenols

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Our main interest is actually the development of new strategies for the valorization of industrial waste, studying new synthetic pathways for preparing biologically active molecules starting from compound of no biological value, such as, in the present case, tyrosol. Tyrosol, a cheap molecule largely present in the waste of olive oil, is a phenol, polluting for the environment, which must be disposed with appropriate costly procedures. Since from several years we are studying the possibility to transform this molecule in value-added compounds. In this context, we are prosecuting our investigation to synthesize polyphenols from tyrosol, exploited a protocol developed in our laboratories consisting of 1) selective and environmentally sustainable bromination of easily available aromatic compounds, 2) methoxylation with MeONa in the presence of CuBr, and 3) eventual demethylation with BBr_3 to obtain the final product. In the last year, we developed the synthesis of benzofuran derivatives, and we are optimizing the procedure. Actually, we are studying a method to obtain, from tyrosol, some compounds with flavonoidic skeleton to develop a strategy to synthesize flavones rarely present in nature, with potential interesting biological properties. All compounds will be prepared in a pure form and with one or more moieties protected as ester, with the intent to prepare pro-drugs, more stable compounds, easily transformed in the active principles by enzymes present in the biological media. All new compounds will be fully characterized and tested for the biological activities, mainly as antioxidants, but also for different types of activities.

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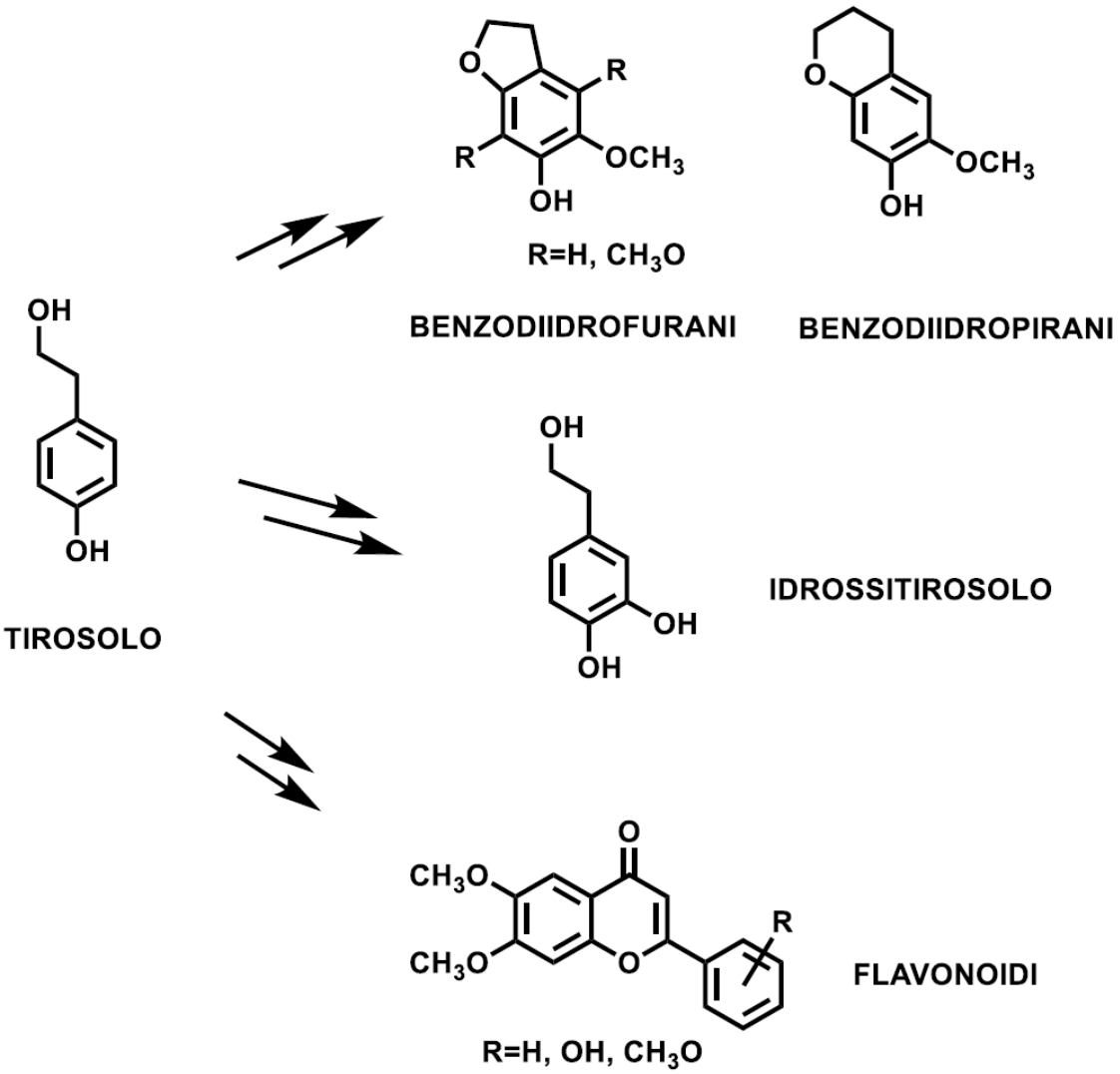
O
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R
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IDROSSITIROSOLO
O
CH3O
CH3O
O
R
FLAVONOIDI
R=H, OH, CH3O

Stereocontrolled synthesis of azasugar D-1-deoxygalattonojrimycin

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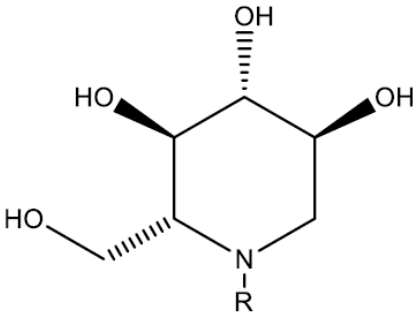
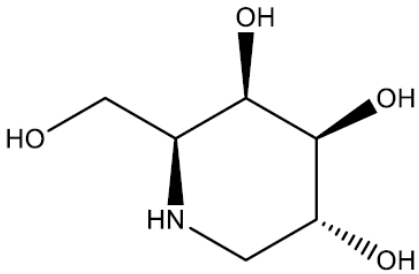
Polyhydroxylated pyrrolidines and piperidines, also known as azasugars or iminosugars, are structural analogues of traditional carbohydrates where the endocyclic oxygen is replaced by a nitrogen atom and, as for sugars, can be found as five terms cycles (azafuranose) and six terms cycles (azapyranose).
Their most valuable property is the ability to inhibit glycosidase and glycosyltransferase enzymes by mimicking the corresponding natural substrates. Therefore these sugar mimics have a tremendous therapeutic potential against a vast array of diseases, from viruses infections to tumoral metastases [1].
Nowadays, two different derivatives of 1-deoxynojirimicin are currently in the market: (miglitol (Glyset®), a drug for the treating of type II diabetes mellitus, and miglustat (Zavesca®) used for the treatment of Gaucher desease. More recently, the European Commission approved the drug 1-deoxygalactonojirimycin, trade name Galafold (formerly known as Amigal), [2] for the treatment of Fabry disease [3], a rare genetic disorder.

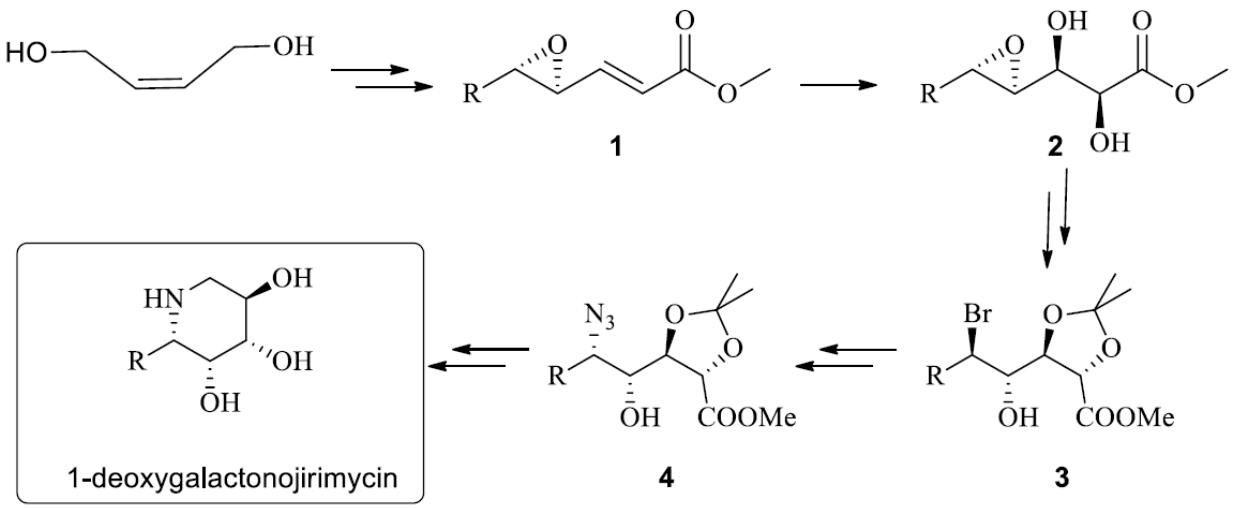


R = H 1-deoxynojirimicin
R = *n*-Bu Zavesca
R = $(CH_2)_2OH$ Glyset



1-deoxygalactonojirimycin
Galafold
(formerly known as Amigal)

Taking advantage of our approach for the synthesis of iminosugar, we designed the stereocontrolled preparation of 1-deoxygalactonojirimycin, starting from the commercially available *cis* 2-butene-1,4-diol.



The key steps of the synthetic pathway are the double diastereoselection in the asymmetric dihydroxylation of chiral vinyl epoxy alcohol [1,4] and the regio- and stereoselective opening of the epoxide [2] using LiBr/Amberlyst [1,5] system, followed by the displacement of bromine by azide ion [5]. Finally, from azido alchol [4], few already optimized reactions allow to achieve (+)-1-deoxygalactonojirimicin in satisfactory yield.

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From formamide to RNA the prebiotic path is tenuous but continuous

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Life is made of the intimate interaction of metabolism and genetics, both built around the chemistry of the most common elements of the Universe (hydrogen, oxygen, nitrogen, carbon).
The origin-of-life quest has long been split in several attitudes exemplified by the aphorisms “genetics-first” or “metabolism-first”. Overstepping the opposition between these approaches by a unitary theoretical and experimental frame, and taking into account energetic, evolutionary, proto-metabolic and ur-environmental aspects, we propose a simple pathway leading to a complete prebiotic reactive system. Specifically, we analyze the synthetic reactions leading from the one-carbon atom compounds HCN and its hydrolyzed form NH_2COH formamide to prebiotically relevant compounds in the presence of catalysts. We observe the formation of all the extant biological nucleic bases, of carboxylic acids, of aminoacids and of condensing agents in the presence of tens of catalysts of terrestrial origin and of 12 meteorites. We also observe in the same chemical frame the formation of cyclic nucleotides and their spontaneous polymerization to oligonucleotides, their terminal ligation yielding longer polymers, a ribozyme activity causing the terminal transfer of nucleotides between in vitro abiotically generated oligomers. In vitro generated oligonucleotides thus automatically increase the chemical information of the system.
This is not to say that from a formamide test-tube one can magically obtain RNA. Numerous hurdles remain and the results are so far very partial. Anyhow: (i) all extant nucleic bases can be abiotically synthesized. (ii) Nucleosides: observed formation of all of them, except guanosine, under proton irradiation. (iii) Phosphorylation: observed formation of cyclic nucleotides from preformed nucleosides. (iv) Polymerization: characterized for 3’,5’cGMP, 3’,5’cAMP, and 3’,5’cCMP.
These results entail that the spontaneous generation of proto-metabolic and proto-genetic systems would have required a not exceedingly complex initial set-up. Rather, it was probably the result of **the interplay between combinatorial chance and thermodynamic necessity of the existing most** abundant atoms.

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Regulation of Vitamin B6 metabolism in bacteria: involvement of the MocR family of pyridoxal phosphate-dependent transcriptional regulators

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Vitamin B6 is an ensemble of six vitamers including pyridoxal, pyridoxamine, pyridoxine and their related 5'-phosphate derivatives (**Fig. 1**). The biologically active form of the vitamin is pyridoxal 5'-phosphate (PLP), which acts as coenzyme in more than 160 distinct enzymatic activities involved in essential metabolic pathways. However, the recent discovery of unsuspected roles of vitamin B6 in many biological processes, ranging from antioxidant activity to regulation of gene expression, has challenged the traditional concept of PLP as being only a cofactor involved in catalysis.

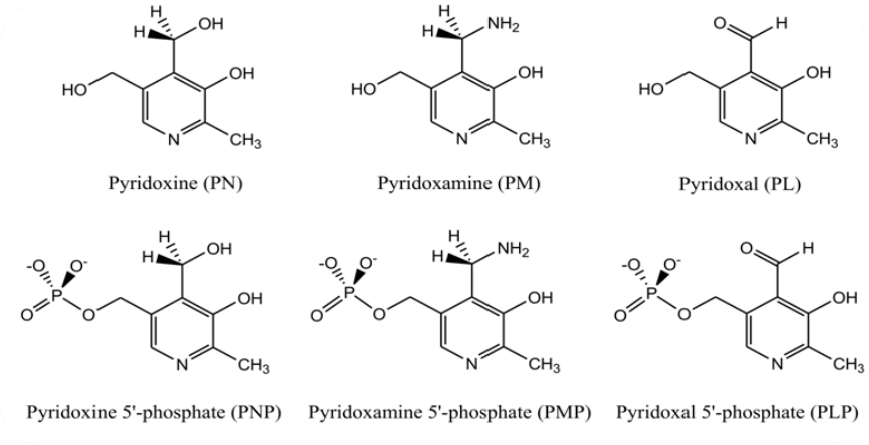

Fig 1. B_6 vitamers.

Although PLP is essential for all living beings, only plants and microorganisms are able to synthesize it *de novo*. All other organisms acquire B6 vitamers from nutrients and protein turnover, and interconvert the different forms using enzymes of the so-called "salvage pathway". Despite the importance of vitamin B6 metabolism, only few investigations focused on the regulation of vitamin B6 biosynthesis and recycling, and in particular very little is known on the regulation of gene expression.
Recently, a new subclass of bacterial transcriptional regulators structurally related to PLP-dependent enzymes, the MocR subfamily of GntR regulators, has been discovered. This is characterized by an N-terminal portion made of a helix-turn-helix motif that binds DNA and a C-terminal domain, called effector-binding and/or oligomerization domain, which is homologous to a specific subfamily of PLP-dependent enzymes. A polypeptide linker that varies in length connects the two domains [**1**]. The solution of the 3D structure of GabR from *Bacillus subtilis* showed that this regulator exists as a domain-swapped homodimer, in which the HTH domain of one subunit interacts with the PLP-binding domain of the other subunit (**Fig 2**).

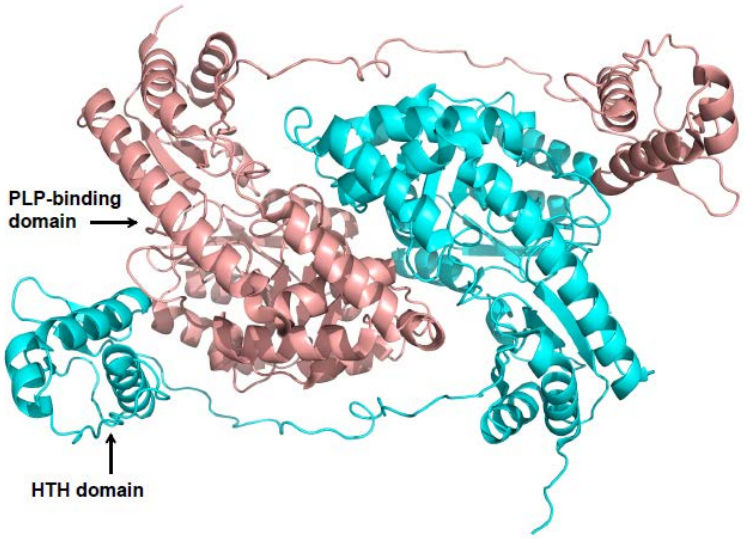

Fig. 2. Crystal structure of GabR from *B. subtilis*

Our research group has been working on MocR regulators for the past three years, using bioinformatic and experimental approaches [1-5]. We analyzed the biochemical and DNA-binding properties of PdxR, a MocR regulator from *Bacillus clausii*, an important probiotic organism used in pharmacological preparations such as Enterogermina, which regulates the biosynthesis of vitamin B6 by binding to its own final product PLP [1]. Recently we characterized PtsJ from *Salmonella typhimurium*, which in the presence of PLP represses the expression of an enzyme involved in PLP salvage pathway [5]. In both cases we studied the structural characteristics of proteins and their transcriptional factor binding sites on DNA, and we suggested models that explain how PdxR and PtsJ regulate transcription of their targets.

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Merosin-deficient Congenital Muscular Dystrophy type 1A (MDC1A): an innovative therapeutic strategy based on Zinc Finger Artificial Transcription Factors (ZF-ATFs)

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MDC1A is a congenital form of neuromuscular disorder caused by recessive mutations in the laminin α2 gene, resulting in the production of a truncated form or complete loss of laminin α2 protein. MDC1A patients typically show severe muscle and neurological dysfunctions.
Laminin α2 (Lama2) is required for the formation of heterotrimeric laminin-211 and laminin-221, which are the major constituents of skeletal muscle basal lamina. Laminin-111 is the predominant isoform in embryonic skeletal muscle development and it is replaced by Laminin-211 in adult skeletal muscle. Supported by the predicted ability of the embryonic laminin α1 isoform (Lama1) to functionally replace Lama2 function, we propose to up-regulate/reactivate Lama1 gene in MDC1A congenital disorder using zinc finger artificial transcription factors (ZF-ATFs). To construct novel ZF-ATF genes, we compared human and mouse Lama1 gene promoters focusing on two highly conserved regions containing stretches with 100% of human/mouse homology. The human Zif268 natural transcription factor was used as gene backbone to produce seven novel Lama1-ZF-ATF genes (LZifs) by introducing only few amino acid substitutions. All LZif genes, were individually cloned in the adeno-associated virus vector pAAV, giving rise to pAAV-LZif-1 to 7. The battery of the pAAV-LZif-1 to 7, renamed: "DO, RE, MI, FA, SOL, LA, SI" were tested to select the best performing gene. The selection criteria were based on their ability to specifically bind Lama1 promoter in the context of native chromatin infrastructure and to re-activate/promote transcription. We are currently analysing the effects of the selected "MI" and "SI" genes in different cell lines, including MDC1A patient-derived myoblasts. These experiments precede the studies in the appropriate MDC1A mouse animal model.

Hippocampal neurons of dystrophic *mdx* mice are less responsive than wild type to acute corticosterone treatment *in vitro*

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Stress induces hypothalamus-pituitary-adrenal axis activation and increase in circulating glucocorticoids (GCs). Major brain target of GC is the hippocampus, in which acute and chronic stressful stimuli induce changes in neuronal activity and synaptic functions, relying on circuit remodeling largely mediated by gene expression modifications. GCs are anti-inflammatories, used as treatment in a variety of pathologies, including the Duchenne Muscular Dystrophy (DMD), a lethal, X-linked disease characterized by muscular wasting consequent to lack of dystrophin (Dp427). As Dp427 is also expressed by several brain regions, including hippocampus, DMD patients experience various neurological disorders. We analyzed whether GC treatment affected hippocampal neuron physiology, with particular attention to modulation of GC receptor (GR) expression. GR mRNA and protein levels were analyzed in hippocampal neuron cultures from E18 WT and dystrophic *mdx* mice, following acute incubation with either 1μM or 10μM corticosterone. In WT mouse neurons, GR mRNA levels significantly increased after both corticosterone treatments, compared to control (vehicle alone). Differently, in *mdx* mouse cells, mRNA levels increased slightly (non-significantly), only after 1μM corticosterone incubation. Protein levels (Western immunoblots), localization (immunocytochemistry) and intensity of immunolabeling of GR and *p*GR (phosphorylated) changed accordingly to mRNA. We hypothesize that *mdx* mouse neurons could be “pre-sensitized” by blood-derived GC of pregnant mothers, afflicted by mild myodegeneration and inflammation. This could interfere with GC signaling *in vitro*, altering GR mRNA synthesis and consequent post-transcriptional modifications.
To extend and to complete the results obtained in vitro we have analyzed Gr gene expression and protein synthesis obtained after acute treatment with corticosterone in hippocampus in vivo.
Moreover, as in *mdx* mice and DMD patients neuronal alterations begin during development, steps of the GC-GR signaling could also be directly affected.

Neurotrophin level changes induced by QMT, a new cognitive-motor training

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Neurotrophins are closely related to stress and well-being. Initially synthesized as precursor proteins, they can influence both developing and mature neural circuits, utilizing distinct receptors to mediate divergent neuronal actions, such as neuronal differentiation, synaptogenesis, and synaptic plasticity. Neuroplasticity implies remodeling of neuronal structures, which in turn involves molecular modifications. Maintaining neuroplasticity, is an important goal, which can be stimulated through training, by activating molecular mechanisms, for example, regulation of growth factors.
The *Quadrato Motor Training* (QMT) is a specifically-structured movement meditation in response to verbal commands, which was found to improve neuronal synchronization and to increase creativity, reflectivity, attention, as well as neuroplasticity. In comparison to other practices, the QMT has the advantage of being a training of relatively short duration (possibly several minutes) and can be practiced in limited spaces. These unique aspects render the QMT a technique warranted for scientific exploration, with the future aim of implementing this technique in various health promoting and educational setups.
The aim of the current study is to examine the link between structural and neurotrophic changes following the QMT, providing novel insights regarding the possible underlying neural and molecular mechanisms.
We analyzed by western blot the protein levels of two neurotrophins, BDNF and NGF, in saliva samples of healthy volunteers who practiced QMT for 1-3 months. The results show that the training induces a decrease of proNGF, and an increase of proBDNF, relative to controls. These molecular changes were correlated with increased creativity, as assessed by the alternate uses task, and with increased cerebellar volume, including synaptogenesis and dendritic arborization, as shown by multimodal magnetic resonance imaging (MRI).
As a preliminary study, we examined the effects of QMT on reading and molecular changes in a 20-years old dyslexic Italian reader, utilizing the one minute reading task and measuring neurotrophins levels. The subject performed the QMT daily for a period of 10 weeks. The reading task as well as the molecular detection were performed before, and following 4 and 10 weeks of treatment. In line with our hypothesis and consistent with our previous results, we detected increased BDNF levels. Improved reading was revealed by reading task and creativity tests. We therefore suggest QMT as an effective training to treat dyslexia in terms not only of improved reading, as was previously demonstrated, but also in terms of molecular change.

Circular RNAs in Autism Spectrum Disorder

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Tight regulation of RNA metabolism is essential for normal brain function. This includes co and post-transcriptional regulation, which are extremely prevalent in neurons. Circular RNAs (circRNAs) are RNAs with a covalently closed loop structure, formed by non-sequential back-splicing of pre-mRNA transcripts. They are stable, well conserved during evolution and highly abundant in neuronal tissues. In the brain circRNAs are often derived from genes specific for neuronal functions, and their expression is controlled during neuronal development and by synaptic plasticity. Although the function of most circRNAs is still elusive, their role in the regulation of synaptic functions during development has been recently proposed. Autism spectrum disorder (ASD) is a common, highly heritable neuro-developmental condition, with a marked genetic heterogeneity. It is characterized by impairments in social interaction and communication, and repetitive behaviors. Evidence indicates that many of the genes that are mutated in ASD are involved in the regulation of synapse development and plasticity. In this work we aim to clarify the role of circRNAs in the aetiology of ASD. By deep RNA profiling, we analyzed circRNAs expression in the hippocampus of the ASD mouse model BTBR T+ Itpr3tf/J (BTBR), compared to C57BL/6J control mice. A number of circRNAs differentially expressed in BTBR mice have been identified. Relevant candidates have been selected and validated in their structure and expression by RNase exonuclease sensitivity and RT-qPCR analysis, respectively. Studies are in progress to characterize the role of selected ASD-related circRNAs in term of biological function and molecular mechanisms of action.

Altered protein *O*-GlcNAcylation profile revealed by proteomics: novel insights on protein signalling mechanisms in Alzheimer Disease

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Background. PET scan analysis have demonstrated the early reduction of cerebral glucose metabolism in AD patients (1) that can make neurons vulnerable to damage *via* several mechanisms including the alteration of the hexosamine biosynthetic pathway (HBP) (2). In turn, defective HBP lead to flawed protein *O*-GlcNAcylation coupled, by a mutual inverse relationship, with the increased protein phosphorylation on Ser/Thr residues. Impaired *O*-GlcNAcylation of Tau and APP have been reported in AD and are closely related with pathology onset and progression (3,4). As well, type 2 diabetes patients show altered GlcNAcylation/phosphorylation balance together with an increased risk to develop AD (5). Therefore, altered protein *O*-GlcNAcylation might represent a link between metabolic defects and AD progression.
Methods. Our study aims to decipher the status of HBP pathway, the role of total *O*-GlcNAcylation reduction and the specific protein targets of altered *O*-GlcNAcylation in brains of 12 months-old 3xTg-AD compared with age-matched wild-type mice. Hence, we analysed: 1) The global O-GlcNAcylated protein levels, 2) level and activity of *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA), the enzymes controlling its cycling; 3) Specific O-GlcNAcylated proteins by 2D proteomic approach coupled with ESI-MS/MS; 4) Tau hyperphosphorylation and O-GlcNAcylation to highlight their mutual relationship.
Results. Our data demonstrated altered enzyme activity and expression levels of OGT and OGA, increased tau phosphorylation and a general decrease of total *O*-GlcNAcylation levels in 12 months-old 3xTg-AD compared to wild-type. Data from proteomics analysis led to the identification of several proteins with differential *O*-GlcNAcylation levels, between transgenic and wild-type animals, which belong to key pathways involved in the progression of AD such as neuronal structure, degradation processes and energy metabolism. Interestingly, the majority of proteins identified by MS analysis show the concomitant alteration of phosphorylation levels, suggesting that the unbalanced *O*-GlcNAcylation/ phosphorylation levels may lead to altered functionality of these proteins and contribute to early cognitive defects of AD.
Conclusions. Our findings may contribute to understand the effects of altered protein *O*-GlcNAcylation profile during AD, identifying novel mechanisms of disease progression related to glucose hypometabolism.

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In situ detection of protein interactions and modifications by proximity-ligation assay

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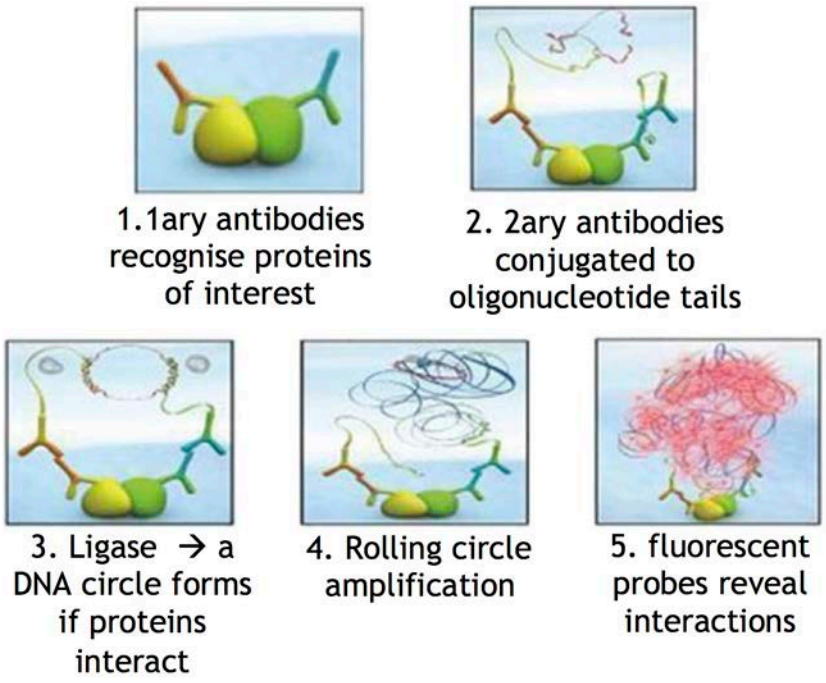
Progress in cell biology largely relies on the development of novel informative imaging techniques, which have revolutionized our understanding of intracellular molecular processes. The proximity ligation assay (PLA) technique bridges imaging and protein methods. It uses antibodies-DNA conjugates to detect protein targets of interest by fluorescence microscopy, and can detect them with spatial and temporal resolution in intact cells (Figure 1). Here we present PLA advances and applications developed in our laboratory to dissect complex processes.

First, we have employed PLA to depict variations in protein-protein interactions (PPIs) in space and time in whole cells. To exemplify this, we present data showing the relocalisation of protein complexes containing nuclear transport factors from the nuclear envelope (NE) in interphase, to the mitotic spindle microtubules and kinetochores in mitosis, and eventually their reassembly with the reforming NE around the decondensing chromatin at mitotic exit (2). The PLA method thus accurately defines the timing of variation for specific PPIs.

Second, PLA can depict protein post-translational modifications (PTMs), even involving quantitatively minor protein fractions that, otherwise, would be difficult to depict by conventional co-immunoprecipitation. PLA also provides information on the temporal window in the life of the cell, and the subcellular site or structure, at which protein PTMs actually take place.

Finally, we have implemented PLA to an automated mode that can be used to validate PPIs identified in large-scale proteomics or interactomics screening. As an example, we show the automated validation of 6 interactions from 273 hits depicted in the interactomic analysis of the importin beta protein during mitosis.

These applications increase the informative power of PLA as an *in situ* "addition to the proteomics toolbox" (2) in studies of protein interactions and post-translational modifications in intact cells.



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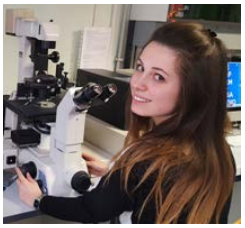
SUMO-conjugation of the Aurora-B kinase is essential during mitosis



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Aurora B is a highly conserved protein kinase with roles in mitosis and meiosis. Aurora B is required to monitor and correct possible errors that may occur in mitosis, when the spindle microtubules (MTs) interact with chromosomal kinetochores (KT). This error monitoring function of Aurora-B is therefore essential to orchestrate balanced chromosome segregation to daughter cells. At later stages, Aurora B also acts in cytokinesis.

Defective Aurora B activity is reported to cause chromosome segregation defects and aberrant cytokinesis, hence originating genetic instability in daughter cells. Both the expression level and the kinase activity of Aurora B are dysregulated in several cancer types. Understanding how Aurora B is itself regulated in a physiological mitosis is therefore a relevant issue.

SUMOylation (conjugation with small ubiquitin-like modifier, or SUMO, oligopeptides) is a post-translational modification of growing importance that modifies the interaction surface of proteins, and hence affects their profile of interaction with protein partners.

Here we show that Aurora B is modified by conjugation with SUMO2/3 oligopeptides. In situ proximity-ligation assays indicate that SUMOylated Aurora B localizes at the MT/KT interface in the prometaphase/metaphase window, suggesting that this modification is implicated in MT/KT attachments. We have constructed stable cell lines that express Aurora B mutants mutagenized in the putative SUMOylation site (SUMO-null mutants). We find that expression of SUMO-null Aurora B significantly alters mitotic progression in cells with an Aurora B-proficient background with a dominant negative effect, and, furthermore, fail to replace the endogenous wild-type Aurora B in an Aurora B-silenced background. These data indicate therefore that the SUMO acceptor site is a previously unrecognized element important for Aurora B function at KTs. These results uncover novel aspects of Aurora-B regulation, important to understand how its dysfunction might affect cell division in cancer cells.

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The Sf3A2 and Prp31 splicing factors play a direct role in mitotic chromosome segregation

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Several RNAi-based screens, including one performed in our laboratory, have led to the identification of many proteins required for chromosome segregation. Surprisingly, these proteins included more than 40 splicing factors (SFs) (Kittler et al., 2004, Nature 432:1036-40; Bjorklund et al., 2006, Nature 439:1009-13; Goshima et al., 2007, Science 316:417-21; Somma et al., 2008, PLoS Genet. 4: e1000126; Neumann et al., 2010, Nature 464:721-7).The splicing machinery comprises more than 150 proteins organized in several complexes (Hofmann et al., 2010, Nucleus 1:447-59). The phenotypes caused by depletion of the SFs mentioned above range from defects in chromosome alignment and/or segregation to failures in cytokinesis. However, it is currently unclear whether SFs are required for splicing of RNAs that encode mitotic proteins or are instead playing direct roles in open mitosis (the mitotic events that occur after breakdown of the nuclear envelope) (Hofmann et al., 2010). We have recently analyzed the mitotic role of Sf3A2 and Prp31 and shown that these SFs functions in open mitosis. RNAi-mediated depletion of these proteins in Drosophila S2 cells resulted in a strong metaphase delay comparable to that caused by depletion of KMN (the kinetochore complex responsible for microtubule attachment). In vivo RNAi produced highly defective spindles also in larval brain cells. Sf3A2 and Prp31 bind the spindle microtubules (MTs) in the vicinity of metaphase chromosomes, as well as purified MTs in vitro. Immunostaining showed that both these SFs are required for recruitment of Ndc80 and Mitch (the Drosophila homologue of Spc24/Spc25) at kinetochores. Consistent with these findings, Sf3A2 and Prp31 directly bind KMN components, such as Ndc80, Nuf2, Mitch. Similar results were obtained in HeLa cells, where siRNA-mediated depletion of SF3A2 and PRP31 reduced Hec1/Ndc80 recruitment at kinetochores and caused a partial metaphase block with misaligned chromosomes. Finally, anti-Sf3A2 or Prp31 antibody injection into wild type Drosophila embryos resulted in severe mitotic defects 3-5 minutes after injection, ruling out the possibility that Sf3A2 and Prp31 disrupt mitosis by compromising the splicing of mitotic RNAs. These results indicate that SFA3 and Prp31 play a conserved mitotic function by mediating the kinetochore-MT interaction in both *Drosophila* and human cells.

Synthesis and biological evaluation as apoptosis-inducing agents of styrylheterocycles analogs of resveratrol

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Background. Apoptosis is a route of cell death induced by a highly regulated intracellular program. An increase in apoptotic activity could lead to neurodegenerative pathologies, whereas a decrease in apoptotic activity could lead to uncontrolled cellular proliferation [1].

Objective. The aim of this research was to synthesize ten styrylheterocycle compounds, analogs of resveratrol, having a thiophene ring substituted in position 2- or 3- and a benzene ring with one or two hydroxy and methoxy groups [2, 3]. The compounds were evaluated for their cytotoxicity and possible apoptotic activity against the U-937 cancer cell line.

Method. The experiments were conducted at 1000 µM, 250 µM, 100 µM, and 50 µM analysing apoptotic activity as % of hypodiploid nuclei. The cytotoxicity was expressed as the concentration of a substance able to inhibit 50% of the metabolic activity in an MTS assay, and as the percentage of cellular death at 1000 µM and 100 µM.

Results and Discussion. Changing the phenyl group in a thienyl group occupying the 2- or 3-position almost always leads to an improvement in apoptosis. In addition, whereas the presence of a hydroxy group makes the molecules more cytotoxic than resveratrol, it also improves apoptosis. However, the substitution of the MeO-group instead of the –OH group in the phenyl ring leads to collapse of the cytotoxicity. This allowed an increase in the analytical concentrations of some tested compounds, verifying that, at 1000 µM, the compounds (E)-3-(4-methoxystyryl)-thiophene (3b) and (E)-3-(3methoxystyryl)-thiophene (3c), showed the highest apoptosis levels (81% and 72%, respectively).

Conclusion. We look forward to synthesizing other heterocyclic molecules to find even more active derivatives endowed with anti-cancer potential.

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Use of Ferritin-Based Metal-Encapsulated Nanocarriers as Anticancer Agents

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The ability of ferritin to bind and deliver metals and metal-based drugs to humanneuroblastoma SH-SY5Y cells was studied. We used heavy chain (H) ferritin-based metal-containing nanocarriers to test whether these constructs, which are able to cross the blood-brain barrier, may be used for the delivery of toxic molecules to brain cells, and to study their effect on the viability and cellular redox homeostasis of human neuroblastoma cells. We show that metal-containing nanocarriers are efficiently captured by SH-SY5Y cells. Iron-containing nanocarriers have a proliferative effect, while silver and cisplatin-encapsulated nanocarriers determine concentration-dependent neuroblastoma cell death. This work is a proof of concept for the use of ferritins for the delivery of toxic molecules to brain tumors.

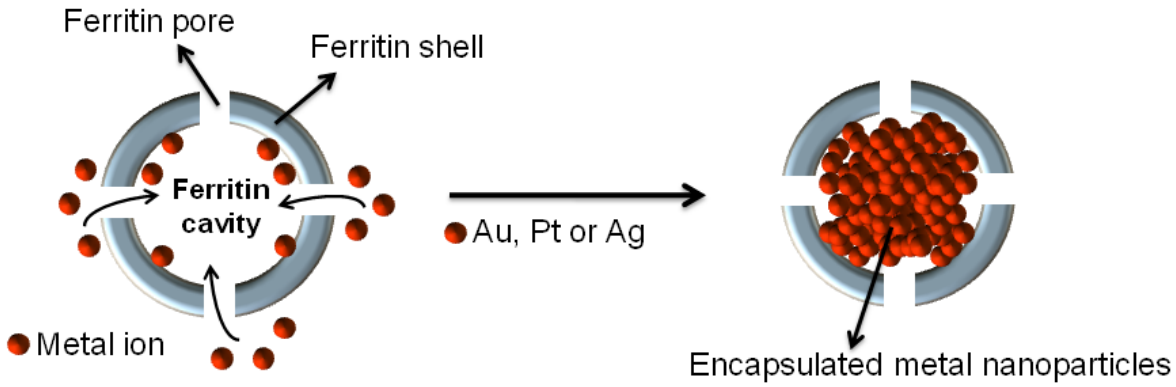

Figure. Summary of the synthesis of metal-containing HFt-based nanocarriers.

Acknowledgments: This work was supported by grants from Ateneo Sapienza 2014 and 2015 (L.M.). "Quality Methods for Design of Experiments in Scientific Research", in the FaReBio di Qualità Project: Quality and Project Management Open Lab qPMO CNR, CNCCS CNR (National Collection of Chemical Compounds and Screening Center 2015–2016), the CNR InterOmics Flagship IBISA project, the Flagship Project Nanomax: "NADINE: NAnotechnology-based Diagnostic In Neurological disease and Experimental oncology", Ministero della Salute "Ricerca Finalizzata" RF2010-131 to G.C. are acknowledged. Associazione Italiana per la Ricerca sul Cancro (AIRC) I.G. Grant 16776 to P.C. is acknowledged. We thank Drs. Valeria de Turris, Italia Anna Asteriti, Andrea Fernandez and Flavia Zanni for their invaluable technical assistance. Live cell imaging experiments were performed at the CNR-IBPM Nikon Reference Centre (Rome, Italy).

Small molecules targeted to the microtubule-Hec1 interaction inhibit cancer cell growth through microtubule stabilization

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Highly Expressed in Cancer protein 1 (Hec1) is a subunit of the kinetochore-associated Ndc80 complex, which ensures proper segregation of sister chromatids at mitosis by mediating the interaction between kinetochores and microtubules. HEC1 mRNA and protein are highly expressed in many malignancies as part of a signature of chromosome instability. These properties renders Hec1 a promising molecular target for developing therapeutic drugs that exert their anticancer activities by producing massive chromosome aneuploidy. A virtual screening study aimed at identifying small molecules able to bind at the Hec1-microtubule interaction domain has selected one positive hit compound and two analogues of the hit with high cytotoxic, pro-apoptotic and anti-mitotic activities. The most cytotoxic analogue (SM15) was shown to produce chromosome segregation defects in cancer cells by inhibiting the correction of erroneous kinetochore-microtubule interactions. Live cell imaging of treated cells demonstrated that mitotic arrest and segregation abnormalities lead to cell death through mitotic catastrophe and that cell death occurred also from interphase. Importantly, SM15 was shown to be more effective in inducing apoptotic cell death in cancer cells as compared to normal ones and effectively reduced tumor growth in a mouse xenograft model by apoptosis induction. Mechanistically, cold-induced microtubule depolymerization experiments demonstrated a hyper-stabilization of both mitotic and interphase microtubules. Molecular dynamics simulations corroborate this finding by showing that SM15 can bind the microtubule surface independently from Hec1 and acts as a stabilizer of both the microtubule and the kinetochore-microtubule interaction. These findings are in line with the results obtained using Surface Plasmon Resonance, in which SM15 was able to bind directly and with high affinity to MTs and (to a lesser extent) to tubulin. Overall, our studies represent a clear proof of principle that microtubule-Hec1 interacting compounds may represent novel powerful anticancer agents.

Chemosentization of sarcoma cells by ITF2357 histone deacetylase inhibitor

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Sarcomas are rare tumors with generally poor prognosis, for which current therapies have shown limited efficacy. Therefore, there is an urgent medical need to develop new treatment options for this particular group of patients. Histone deacetylase inhibitors (HDACi) are emerging as a prominent class of therapeutic agents for several cancers; however, little is known about HDACi activity in sarcomas. By using established and patients-derived sarcoma cells with different subtypes, we showed that ITF2357 potently inhibited *in vitro* survival in a p53 independent manner. ITF2357-mediated cell death implied the activation of mitochondrial apoptosis, as attested by upregulation of pro-apoptotic BH3-only proteins and a caspases-dependent mechanism. ITF2357 also induced a canonical-autophagic process, which protected sarcoma cells from apoptotic cell death. ITF2357 activates Forkhead box (FOXO) 1 and 3a transcription factors and its downstream target genes however, silencing of both FOXO1 and 3a did not protect sarcoma cells against ITF2357 induced apoptosis and upregulated FOXO4 and 6. Notably, ITF2357 synergized with doxorubicin to induce cell death of established and patient-derived sarcoma cells. Furthermore, combination treatment strongly impaired xenograft tumor growth *in vivo,* when compared to single treatment, suggesting that combination of ITF2357 with Doxorubicin have the potential to enhance sensitization in different preclinical models of sarcoma. Overall, our study highlights the therapeutic potential of ITF2357 for the treatment of bone and soft tissue sarcoma alone or in rational combination therapies.

Nitric oxide markedly affects glioblastoma stem cells' phenotype and differentiation capacity

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Glioblastoma (GBM) is the most common form of malignant brain tumor. It features local cancer stem cells (glioblastoma stem cells, GSCs) responsible for enhanced resistance to therapies and tumor recurrence. One potential approach to eradicate GSCs is to force these cells to undergo terminal differentiation. Therefore, the role of differentiation-inducing drugs as therapeutic agents is receiving increasing attention.
Nitric oxide (NO) is and intercellular and intracellular signaling molecule in the brain and is involved in neural development. It plays also key roles in GBM pathophysiology as it is implicated in induction of apoptosis, radio- and chemosensitization.
We investigated the effects of Diethylenetriamine/Nitric oxide adduct (DETA/NO) on patient-derived GSCs, and particularly NO influence on neurospheres generation as well as its pro-differentiation ability.
GSCs grown in the presence of 100 μM NO showed a dramatic inhibition of their self-renewal capacity associated with prevented cell proliferation, as evaluated along a time course up to 21 days. Consistently, the stemness markers SOX2, OCT3/4 and OLIG2 showed an early, time- and dose-dependent decrease of expression.
We investigated also NO influence on serum-induced GSCs' differentiation. Interestingly, after a period of reduced cell proliferation rate, due to the shift from floating stem-like cells to adherent differentiating cells, GSCs grown in serum-supplemented medium acquired the ability to proliferate very quickly, as evaluated up to 21 days. A fraction of these cells exhibited the morphology of immature neuronal cells. On the contrary, NO induced a dramatic slowdown of the proliferation rate and the morphology typical of mature bipolar neurons. These changes were paralleled by changes at the molecular level, as serum-induced GSCs' differentiation was associated to a strong reduction of the stemness markers, further enhanced by NO exposure. Moreover, the weak expression of βIII-tubulin, marker of neuronal differentiation, in the cells grown in the presence of serum was strongly increased by NO exposure. However, the persistent expression of GFAP indicated the presence also of a subpopulation of astrocytes.
Histone post-translational modifications play a major role in the organization of chromatin structure and subsequent regulation of gene expression. The analysis of a possible epigenetic role of NO unveiled that NO exposure reduced the level of acetylation of lys9 on histone H3 in GSCs and accentuated serum-dependent deacetylation. The use of specific inhibitors of different classes of Histone deacetylases (HDACs) suggested that NO effect could be mediated by HDAC1 and/or 2.
Taken together, our findings show that NO prevents the stem-like phenotype of the GSCs subpopulation whereas it steers GSCs grown in serum-induced differentiation conditions mainly towards terminally differentiated neuronal cells, which might be a novel therapeutic approach for brain tumors.

Inflammatory microenvironment and human papillomavirus-induced carcinogenesis

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Introduction. The connection between chronic inflammation and risk of cancer has been supported by several studies. The development of cancer might be a process driven by the presence of a specific combination of inflammatory mediators, including cytokines, chemokines and enzymes, in the tumor microenvironment. Virus-induced tumors, like HPV-induced Squamous Cell Carcinomas, represent a paradigmatic example of the interplay between inflammation, as integral part of the innate antiviral response, and malignant transformation.

Methods. The tumorigenic role of inflammatory mediators in HPV^+ cells has been analyzed into supernatant of primary Human Foreskin Keratinocytes (HFK) and keratinocytes transduced by E6 and E7 from mucosal (HPV-16) or cutaneous (HPV-38) genotypes using Human Chemokine Antibody Array - Membrane (Abcam) and inflammatory Multi-Analyte ELISArray Kit (Qiagen). Moreover, real time RT-PCR has been performed to analyze a selection of such inflammatory mediators in these cellular systems. The specific involvement of the viral proteins has been analyzed by silencing experiments with E6/E7 siRNA.

Results. Gro, Gro-α and IL-8 are downregulated, while Angiogenin is slightly upregulated in both the supernatants of K16 and K38 compared to HFK. IP-10, RANTES and TIMP-1 are upregulated only in K16, while MCP-1 is upregulated in K38 supernatant. A panel of genes has been analyzed to confirm the specific inflammatory molecular landscape of K16 and K38. IL-1α, IL-1β, IL-6, TNF-α, MCP-1, MIP-3α, CTACK, IL-8, Gro-α, Gro-β, Gro-γ are all downregulated in both K16 and K38 cells, while IP-10 and RANTES appeared to be slightly upregulated in K16. Silencing experiments of E6 and E7 in K16 cells confirmed the specificity of these effects.

Conclusions. Our results suggest that the expression of HPV oncoproteins allows the modification of the tumor microenvironment through the synthesis and release of specific pro-inflammatory cytokines and chemokines leading to the interference with the leucocytes trafficking and/or a better tumor growth and infiltration.

The role of inflammatory microenvironment in the HPV-induced carcinogenesis is addressed, with a specific focus on the involvement of the immune molecules and microRNAs as well as their delivery through the microvesicle cargo possibly correlated to the different HPV genotype.

GM-CSF inhibits c-kit and SCF expression by Dendritic Cells

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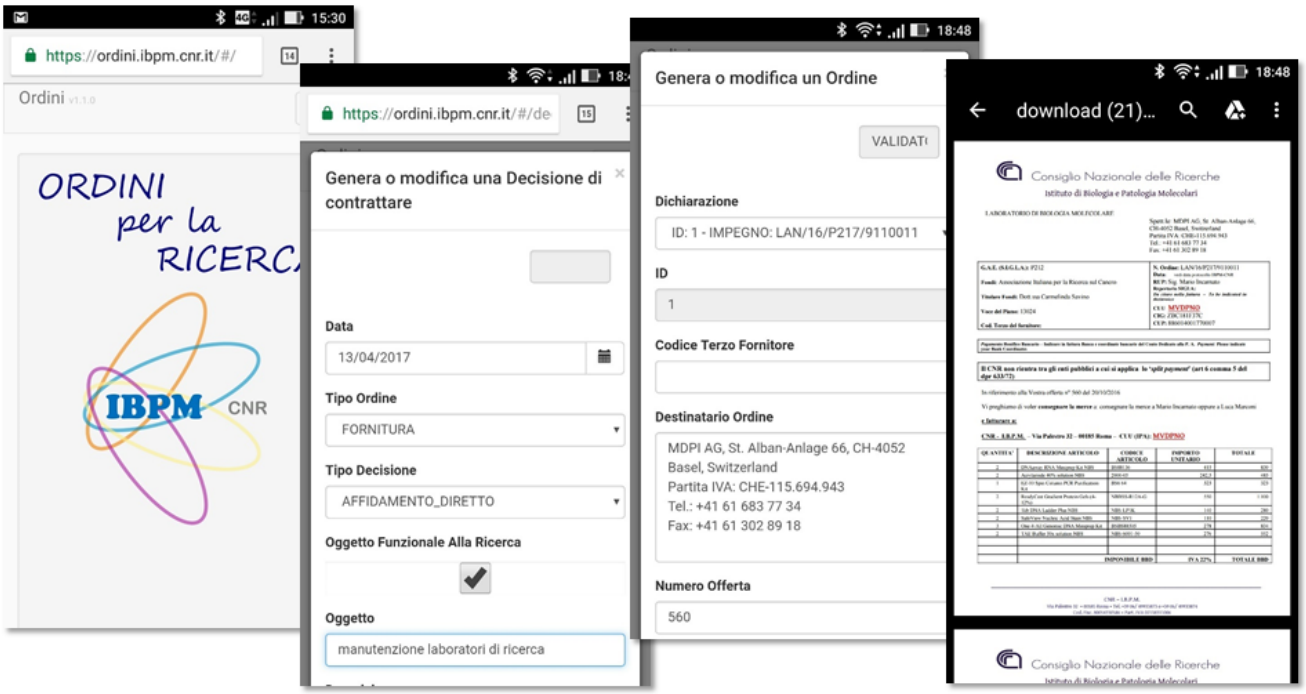
Stem Cell Factor (SCF), the ligand of c-kit, is a key cytokine for hematopoiesis. Hematopoietic precursors express c-kit, whereas differentiated cells of hematopoietic lineage are negative for this receptor, with the exception of NK cells, mast cells and a few others. While it has long been recognized that dendritic cells (DCs) can express c-kit, several questions remain concerning the SCF/c-kit axis in DCs. This is particularly relevant for DCs found in those organs wherein SCF is highly expressed, including the bone marrow (BM). We characterized c-kit expression by cDCs from BM, and demonstrated a higher proportion of c-kit$^+$ cells among cDC1s than cDC2s in both humans and mice, whereas similar levels of c-kit expression were observed in cDC1s and cDC2s from mouse spleen. To further study c-kit regulation, DCs were generated with GM-CSF from mouse BM, a widely used protocol. CD11c$^+$ cells were purified from pooled non-adherent and slightly adherent cells collected after 7 days of culture, thus obtaining highly purified BM derived DCs (BMdDCs). BMdDCs contained a small fraction of c-kit$^+$ cells, and by re-plating them for 2 days with GM-CSF we obtained a homogeneous population of c-kit$^+$ CD40^{hi}MHCII^{hi} cells. Not only did BMdDCs express c-kit, but they also produced SCF, and both were striking up-regulated if GM-CSF was omitted after re-plating. Furthermore, a small but significant reduction in BMdDC survival was observed upon SCF silencing. Incubation of BMdDCs with SCF did not modulate antigen presentation ability of these cells, nor it did regulate their membrane expression of the chemokine receptor CXCR4. We conclude that the SCF/c-kit mediated pro-survival circuit may have been overlooked because of the prominent use of GM-CSF in DC cultures *in vitro*, including those human DC cultures destined for the clinics. We speculate that DCs more prominently rely on SCF *in vivo* in some microenvironments, with potential implications for Graft Versus Host Disease (GVHD) and anti-tumor immunity.

Order Management System Software Project

Mario Incarnato

CNR–National Research Council of Italy, Institute of Molecular Biology and Pathology, Rome, Italy

The software application aims to provide support for the drafting and management of purchase orders for equipment and services useful to research and to institute administrative activities. The application simplifies the administrative process flow related to orders, from their initiation to the various intermediate stages, until completion and sending to the supplier.
Gathering all necessary requirements led to the definition of the main subjects using the application, being the Compiler, the Funds Manager, the Administrative Representative and the Director.



The order application process typically begins by filling out a decision to be negotiated, so the application provides an interactive guide function, based on the default standard MS WORD template of the institute, where the Compiler inserts the required data; a PDF document is then produced, validated and automatically available for the next stage, the completion of the declaration of the RUP. Also in this case, the application facilitates the compilation and production of the resulting PDF document, carried out by the Funds Manager.
The requested order is completed by the Compiler, who through another guided interactive function, also based on the institute's standard MS WORD template, inserting

order data and producing another PDF document.
The workflow is controlled by the Administrative Representative, who may reject the submitted documents, providing reasons, for example requesting correction of details, or validate and accept the documents.
The next step is determined by the final validation of the Director, after which the documents can be forwarded to computer protocol and the order can be sent to the supplier.
The application is created with open source technologies and frameworks, including Java 8, Spring Framework, AngularJS 1.5.8, Bootstrap 3.3.7 and 2.4 Elasticsearch, combined together by JHipster generator 3.12.2.

Remarks

Prof. Clara Balsano, Director of IBPM-CNR

It's a pleasure for me to present for the second year a synthesis of the annual IBPM meeting. Besides an overview of the main research programs and technology transfers of the institute, this year we involved researchers of other research institutions (e.g. Tor Vergata University, Regina Elena Cancer Institute), stakeholders and journalists. The IBPM consists of 66 Researchers, plus 16 associated researchers and 5 administrative personnel.

Despite the national economic difficulties to funds dedicated to basic research, the scientific productivity of the Institute has exponentially improved year by year. From the 2012, as Scientific Director, I contributed to a noticeable scientific growth of younger researchers, who became self-sufficient creating new interests and patents of great scientific value. As already reported in previous years' reports, the Institute has successfully developed translational science even through virtuous collaboration between basic and clinical scientists.

In particular, we produced, in 2014, 69 scientific works with 281 of Impact Factor (IF); in 2015, 82 scientific works with 362 of IF and, in 2016, 112 scientific works with 482of IF. The evaluation of the CNR Institutes, carried out by CNR in 2014, report a higher Institute gaining 2 points in the graded list if compared to that of2009.The average H-index of IBPM researchers is 19.5, confirming the high scientific value of the scientific products and confirming the scientific international excellence of the researchers of the Institute.

The presence, in the Auditorium, of Professor Giuseppe Novelli, the Rector Magnificus of the Tor Vergata University of Rome, and of Doctor Gennaro Ciliberto, scientific director of the Regina Elena Cancer Institute, is proof of the close and virtuous scientific relationship which exists between the IBPM Institute and the distinguished Research Institutes present in the area of Rome.

I know Prof Giuseppe Novelli from a long time, he contributed to the discovery of several genes responsible for genetic human diseases, such as Laron syndrome (Laron nanism or somatotropin alteration), and recently the genes responsible for psoriasis and myocardial infarction. Current studies are geared towards the treatment of hereditary diseases and the development of new drugs and pharmacogenetics.

I’m mostly delighted to highlight his recent initiatives as Rector. He has two great merits:

1. The "refounding" of Tor Vergata's mission, predominantly dedicated to "sustainable development", as outlined by the 17 goals for sustainable development set by the United Nations in September 2015: eradicating poverty, ending hunger, healthy life, quality of the education, gender equality, access to water, clean and sustainable energy, labor and economic growth, innovation and infrastructure, equality between nations, sustainable cities, sustainable consumption, halting climate change, protecting the sea, protecting biodiversity, peace and justice. This initiative has seen a first great time of cultural and social aggregation in the "Italian Alliance for Sustainable Development", founded in Tor Vergata and attended by dozens of cultural institutions and research organizations.

2. The second strategic initiative addresses the Second University of Rome as a place of innovation and technological transfer, incubation of ideas and talents.

Another illustrious personality participates to the meeting: Prof. Gennaro Ciliberto. He became scientific director of IRCSS Regina Elena Cancer Institute in December 2016 for the next five years. The Regina Elena Cancer Institute has recently been accredited by the Organization of European Cancer Institutes (OECI), that It is a network composed by 70 of the most important cancer research and care centers in Europe. Gennaro Ciliberto is Full Professor of Molecular Biology and has over 25 years of experience in managing research at the IRBM research center (Merck Sharp and Dohme). In addition, up to 2016 he was appointed as Scientific Director of IRCSS National Cancer Institute "G. Pascale" in Naples.

The large participation of researchers, both young and old, belonging to the Institute and other prestigious research institutions has allowed for a fruitful exchange of information and experiences. We had a real positive feedback from the last year meeting and, this year again, we succeeded in creating the basis for new networks of excellence, by connecting an important scientific critical mass to provide all the participants with the opportunity to share ideas, hoping, in the near future, for a further qualitative leap in the activities of IBPM’s research. Those who do research are well aware of the significance of belonging to a community and know the importance of team effort in order to achieve a common goal.

Some of the researchers of the Institute have presented their latest research belonging a wide range of scientific domains. The presence of multifaceted and complementary scientific competencies has attracted considerable financial and human

resources. The ability to use multidisciplinary approaches that integrate biochemistry, molecular and cellular biology, genetics, bioinformatics and chemistry makes IBPM, among the DSB-related institutes, the most promising Institute for the development of New molecules for "target therapy". In addition, the wide opportunities for training and sharing of know-how and knowledge with the "Sapienza" University of Rome are a major element of attractiveness for young researchers. Accordingly, of the 81 winners of the last competitions at T.I., two Dr. Daniela Trisciuoglio and Dr. Allegra Via have chosen to join our Institute.

IBPM is at the forefront in the study of regulation of gene expression and in the study of the molecular mechanisms involved in the pathogenesis of cancer, chronic neurodegenerative, metabolic and infectious pathologies. In this direction is the recent signature of the agreement between the IBPM and the Cluster of Health Innovation and Community (C.H.I.CO.). C.H.I.C.O. is the first cluster of health in Lazio Region, it is a non-profit private organization focused on life sciences and health sectors. Cluster members are Public Health Companies, Universities, Research Organizations, Hospitals and Companies in the Pharmaceutical, Biomedical, ITC, Functional Food, Agro-Food Industries. The mission of C.H.I.CO. is to maximize the global competitiveness of Cluster members through the development of relationships and promoting cooperation for innovative projects. The studies developed within the Institute aim to create the

underlying assumptions for a basic research that could easily have an industrial, biotechnological, pharmacological and/or food quality application. Studies are ongoing on: effective agents to counteract the onset and progression of neurodegenerative diseases, metabolic disorders, and viral infections (e.g. chemical chaperones with suppressor activity on neuronal neurodegeneration; new copper chelants effective in treating Metabolic syndrome, AAV mediated delivery of artificial transcription factors) and molecules with proven anti-tumor efficacy (e.g., intelligent Nano-transporters, as human ferritin-based drugs).

Added value is the presence of a Biocrystal facility that supports scientific groups interested in developing aspects of structural biology. This platform is able to provide advanced consulting services for the protein expression, their purification, characterization, crystallization and determination of the structure. The bioimaging platform, on the other hand, offers to the IBPM researchers the possibility to carry out dynamic studies, with strong space-time and dynamic components, for qualitative and quantitative analysis of cellular structures, and is therefore of great utility for biological studies.

In these years, the Institute has also shown great scientific liveliness, increasing the capacity to attract external funding (Fig. 1) by achieving external sources mainly from "charities", such as: Italian Association for Cancer Research (AIRC) and the Telethon Foundation.

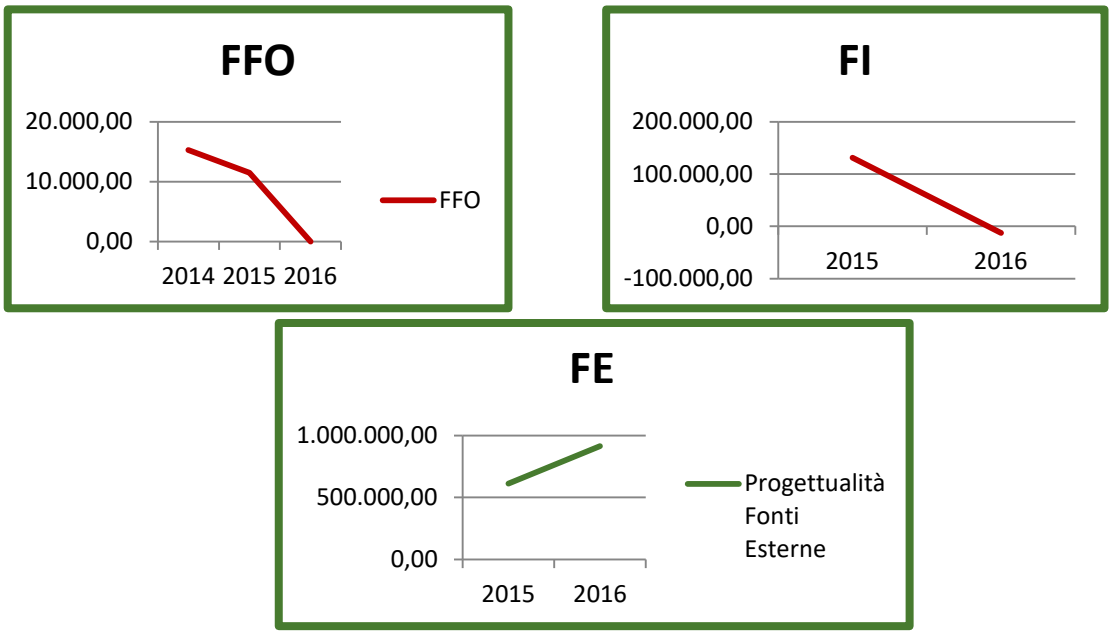


Fig. 1. **FFO:** Ordinary funding fund; **FI:** Internal Funds; **FE:** External Funds.

The success obtained is also a sign of the prevailing widespread recognition that

the knowledge of the mechanisms underlying life has not only an intrinsic cognitive value, but has also many potential and significant applications in biotechnology and biomedical technologies, all the most significant research institutions in developed countries have decided to fund and significantly support the current called "Life Sciences". It is necessary that the Italian government invests in basic research mainframes, which are the bases of applied research. Basic research has important driving function: safeguarding health, safeguarding the environment, working for peace and quality of life. The latter are strategic goals indicated by ONU in 2015. Hence, we have a concrete need to define a percentage of the overall European taxation that have to be devoted to fundamental research assets.

Let me make few final remarks about the third mission. The event organized today is the demonstration of how I consider important what is now called the “third mission”. The third mission is the opening of science to the socio-economic context through the valorization and transfer of knowledge, so a research Institute, such as IBPM, must favor the direct application of basic research, supporting the realization of patents, participating to incubators and consortia dedicated to the technological transfer, in order to enhance knowledge and contribute to the social, cultural and economic development of the country. The idea is to mix cards to play a different game. What Vivaldi would have called: The Cement of Harmony and Invention. Where the real parts of musical writing affect each other, generating a dynamic and meaningful effect. Even the last violin of the last row can teach us something, perhaps because of its particular real experience that falls together, modifying it. A research organization must therefore be a place of innovation and technological transfer, incubating ideas and talents, but also seeking to make a substantial contribution to the social and economic model of society.

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