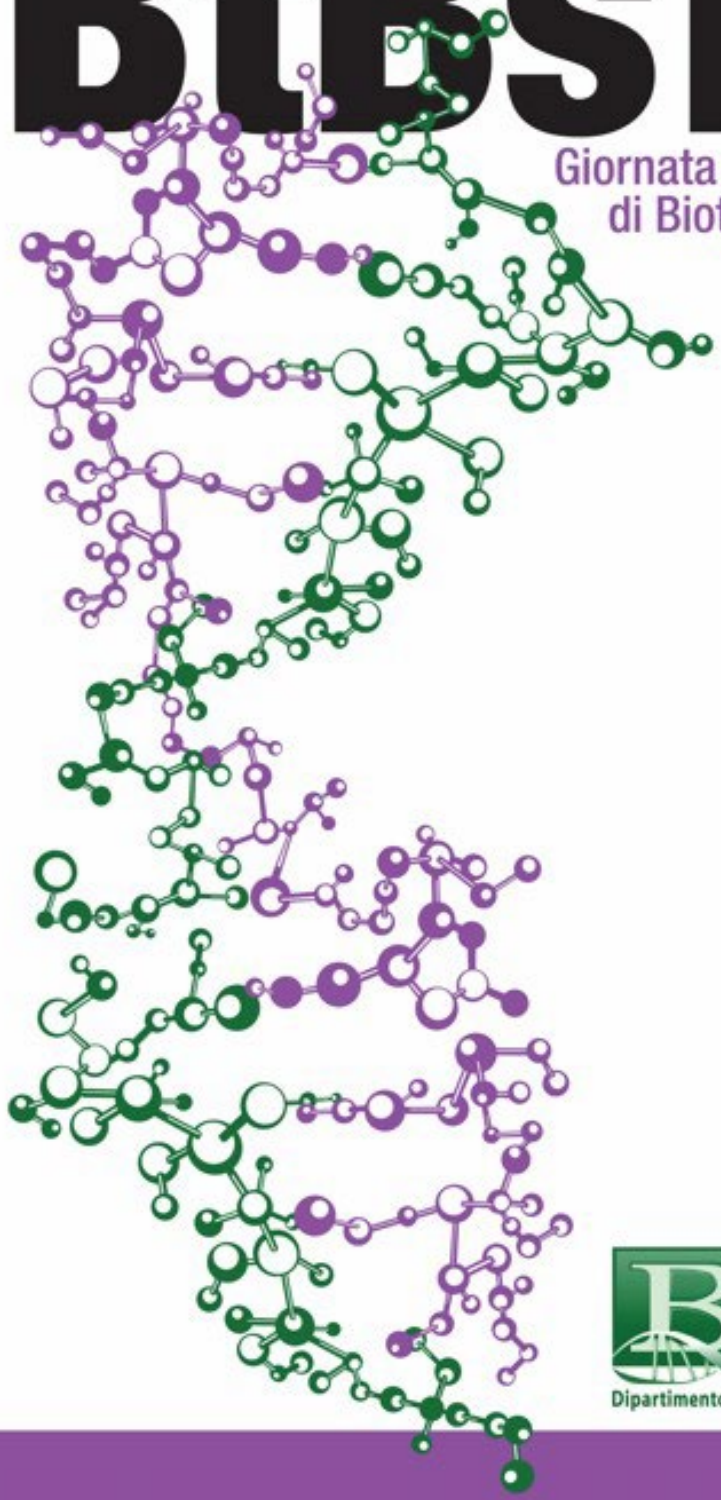
2 DICEMBRE 2014

BtBs Day

Giornata del Dipartimento di Biotecnologie e Bioscienze



BtBs
Dipartimento di Biotecnologie e Bioscienze

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

Program

h 09:00 REGISTRATION

h 09:30 OPENING: **Marina Lotti** - BtBs Department Director

Chairs: Cristina Fugazza, Diego Bonetti

h 09:45 **Guglielmina Nadia Ranzani** – Biology and Biotechnology Department – University of Pavia **"The molecular genetics of colorectal cancer: a growing complexity"**

h 10:30 **Maurizio Casiraghi** – Macroarea: Biodiversity, marine science, and food research **"Influential passengers: monitoring invasive species through High Throughput DNA Sequencing during EXPO2015"**

h 11:00 Coffee break

h 11:30 **Luca De Gioia** – Macroarea: Industrial and synthetic biotechnology **"Molecular design and synthetic biology"**

h 12:00 **Marco Vanoni** – Macroarea: Systems biology **"An integrated multi-scale approach to the study of cell growth, cell cycle and metabolism in yeast"**

h 12:30 **Silvia Nicolis** – Macroarea: Genetic and molecular mechanisms in cellular biology and pathology: implications for human disease **"Genome-wide perspectives on the molecular functions of the Sox2 transcription factor in brain-derived neural stem cells: a role in genome architecture?"**

h 13:00 Lunch break and Poster session

Chairs: Carlo Santambrogio, Sara Mercurio

h 15:00 **Francesco Nicotra** – Macroarea: Nanobiotechnologies for preclinical studies **"Nanobiotechnology in BtBs"**

h 15:30 **Antonio Zaza** – Macroarea: Target identification and drug development **"Molecular and cellular mechanisms for clinical problems. The case of cardiac arrhythmias"**

Selected abstracts

h 16:00 **Lorenzo Gesuita** – "Could the trascription factor Sox2 have a role in arealization of the neocortex?"

h 16:10 **Sara Bottes** – "Characterization of the role of the transcription factor Sox2 in cerebellum development by means of conditional knock out."

h 16:20 **Massimo Labra** – **"BtBs for EXPO"**

h 16:40 **Poster prize and Closing Round Table:** S. Doglia, P. Fantucci, G. Lucchini, A. Zullini

h 17:10 Aperitif

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ORGANIZING COMMITTEE

btbsday@unimib.it

Reinaldo Alvarez

Diego Bonetti

Sara Bottes

Corinne Cassani

Rebecca Favaro

Matilde Forcella

Cristina Fugazza

Lorenzo Gesuita

Carolina Lenzken

Nicola Manfrini

Marina Martina

Alessandra Martorana

Sara Mercurio

Valentina Pastori

Laura Russo

Carlo Santambrogio

Paola Sperandeo

Camilla Trovesi

The organizing committee thanks Prof. Marina Lotti, Department director, for the opportunity to organize this event.

Thanks to Anastasia Sguera and Enza Scarica for their contribute.

Abstract

Research groups

RESEARCH AREA

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

The area scientific contents concern the definition of innovative methodologies for the investigation and the potential treatment of some classes of pathologies with high impact on public health and often linked to aging. In particular, our attention will be concentrated on pathologies such as cancer, chronic and acute inflammation, neurodegenerative diseases and regenerative medicine. In this context, nanotechnologies together with supramolecular (bio)chemistry, synthetic chemistry, and bioinformatics offer new potentialities i) to study biological mechanisms involved in disease etiology and pathogenesis; ii) to improve the possibility of early diagnosis; iii) to develop new therapeutic approaches based on specific cellular and subcellular drug delivery to interfere with the functionality of disease associated target genes; iiii) to develop new nanostructured biomaterials.

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

Study of biocompatible materials for applications in contactology and nervous system engineering

GROUP LEADER
Lecchi Marzia Maria
marzia.lecchi1@unimib.it

RESEARCH TEAM
Pastori V.

In Ocular Physiology Laboratory we are developing two different research projects: morphological and functional study of neuronal differentiation on extracellular matrices by immunofluorescence and patch clamp analysis, and development of biocompatible contact lenses able to counteract oxidative stress symptoms of ocular pathologies.

F11 cells (mouse neuroblastoma N18TG-2 x rat DRG) are plated on carbohydrate-functionalized matrices (from Cipolla's lab) to study if the substrates are able to drive neuronal differentiation. Neurite outgrowth, observed by immunofluorescence, may be a morphologic manifestation of differentiation, therefore in our laboratory we verify the acquisition of specialized neuronal properties also by functional analysis. The electrophysiological properties of cells are analysed by the patch-clamp technique in the whole-cell configuration. Voltage protocols are applied to measure sodium and potassium current amplitudes. Switching the system to the current-clamp mode we measure the resting membrane potential and we monitor the electrical activity. Undifferentiated cells show slow depolarisations which are not able to reach 0 mV, whereas mature action potentials are registered in differentiated cells.

An important goal for ocular medicine is the development of therapeutic contact lenses able to release drugs directly onto ocular surface. Alterations in antioxidant machineries are involved in many ocular pathologies. Lactoferrin is an iron-binding glycoprotein, present in tears and milk, endowed with different physiological functions such as antimicrobial and antioxidant activity. In our laboratory we study the capability of different soft contact lenses to adsorb and release lactoferrin to restore cellular viability in oxidative stress conditions. Different types of contact lenses are loaded with lactoferrin and then incubated with Human Corneal Epithelial primary Cells. After oxidative stress induction with H2O2 cell viability is evaluated.

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

Beyond the glycoworld

GROUP LEADER
Cipolla Laura
laura.cipolla@unimib.it

RESEARCH TEAM
Russo L., Bini D., Sgambato A., Calloni I., Origgi D., Achilli S., Vento S., Corsaro G., Guizzardi R.

Carbohydrates are one of four major groups of biologically important macromolecules that can be found in all forms of life. They have many biochemical, structural, and functional features that could provide a number of evolutionary benefits or even stimulate or enhance some evolutionary events [1]. Through advances in physical and biological chemistry, the glycobiology field now has the tools necessary to decipher the sequence and the structure of cell- and protein-associated glycans. Furthermore, cellular biologists and chemists have established novel ways in which we can alter and exploit glycan structure function. As this field has matured, it has become obvious that the biomedical community can no longer ignore the details of glycosylation. Glycans are ubiquitous and the ability to understand and control their functions are going to be vital to pioneering future biological and therapeutic breakthroughs [2]. With this challenge in mind the research strategies of Prof. Cipolla group focuses the core activity on a redefined approach to engineer glycan components for biomedical purposes that has emerged from the integration of carbohydrate chemistry, chemical biology, and glycobiology. These include the use of glycans themselves as therapeutic molecules as well as engineering protein and Extracellular Matrix signals to suit multifaced biomedical appllications. Following these general subjects we are working on: i) new synthetic approaches to design and synthetise protein interactors (i.e inhibitors or activators) with antibacterial and antitumoral activity, ii) the syntesis of glycostructures (dendrons and dendrimers) to present specific glycidic epitopes able to interact with cell surfaces receptors to study and modulate their biological activity. iii) new smart matrices (i.e. ECM proteins and synthetic hydrogel and biomaterials) decorated with bioactive carbohydrates to study cell differentiation in tissue engineering applications, in pathological and healthy states.

[1] G. Lauc Frontiers in Genetics? 2014, 5,1
[2]C. Bertozzi et al Chemistry & Biology 2014, 21,16

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

Nanobiolab activities

GROUP LEADER
Prosperi Davide
davide.prosperi@unimib.it

RESEARCH TEAM
Tortora P., Prosperi D., Colombo M., Avvakumova S., Verderio P., Galbiati E., Peneranda Avila J., Pandolfi L., Santini B., Bellini M., Collico V., Riva B., Alessio G., Lattuada M., Rozek E., Rizzuto M. A., Lamolinara A., Podini M., Salvioni L.

The Nanobiolab aim at investigating the potential of biofunctionalized colloidal, polymer-based and biomimetic nanoparticles for biomedical application. In particular, the major areas of research are:

TUMOR TARGETING: DIAGNOSTIC AND THERAPY. The development (synthesis, characterization and functionalization with biomolecules) of novel hybrid nanoparticles consisting of a magnetic core, useful as MRI contrast agent, and an organic shell responsible for the cell receptor targeting action. On the other hand, drug-loaded nanoparticles can be used to target selective cancer cells resulting in the localization of the therapeutic activity, which should strongly reduce the typical side-effects usually encountered with conventional chemotherapeutics.

ANTIVIRAL THERAPEUTICS. Nanotechnology has potential in HIV treatment by two approaches: 1) improving the pharmacokinetic properties of antiretroviral drugs, and 2) assisting drugs to cross the biological barriers (e.g., the blood brain barrier) to target the virus reservoirs.

INFLAMMATORY DISEASES. Bioengineered nanoparticles can be developed to localize, monitor and quantify the early stages of inflammatory bowel diseases (IBDs), particularly Crohn disease and ulcerative colitis inflammatory diseases, and to treat aggressive inflammatory disorders including IBDs, rheumatoid arthritis, transplant rejection, edema, sepsis, and other inflammatory conditions.

INNOVATIVE WAYS TO DELIVER NPS ALTERNATIVE TO THE TRADITIONAL INTRAVENOUS ROUTES OF ADMINISTRATION. In vivo investigation of topical, oral and intranasal administration as promising non-invasive delivery options especially for a regional and/or local effect, improving the patient compliance, improving the pharmacokinetics of degradable peptides and proteins, and reducing the frequency of administration.

COSMETIC APPLICATION. Synthesis and application of Silver and TiO2 NPs as alternative antibacterial agents to toxic parabens in cosmetics.

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

Native Mass Spectrometry for Structural Proteomics

GROUP LEADER
Grandori Rita
rita.grandori@unimib.it

RESEARCH TEAM
Santambrogio C.(Post-Doc), D'Urzo A.(Technician)
External collaborations:
Sobott F. (Antwerpen), Carloni P. (Jülich), Longhi S. (Marseille), Uversky V. (Tampa, FL), Bolognesi M. (Milano), Scoles G. (Udine), Molinari H. (Verona), Zambelli B. (Bologna), Legname G. (Trieste), Cappelletti G. (Milano)

In the last two decades electrospray-ionization mass spectrometry (ESI-MS) has become a reliable and robust tool in structural biology. In particular, "native MS" enables the analysis of intact proteins preserving non-covalent interactions and, therefore, represents a powerful method to elucidate macromolecular architectures in dynamic and heterogeneous systems. In our laboratory, native MS is used together with other biochemical and biophysical approaches, for protein conformational and binding studies.

A first line of research focuses on intrinsically disordered proteins (IDPs), for structural characterization of the conformational ensemble and its responses to mutations and environmental changes. Current investigation involves the following proteins: alpha-synuclein, Sic1, NTAIL, UreG and Ataxin3.

A second line of research focuses on protein-ligand and protein-protein interactions, for structural characterization of the non-covalent complexes, with a particular interest in the procedure for assessing the specificity of the interactions and their structural implications. Current investigation involves the following proteins: fatty-acid binding protein (FABP), maltose-binding protein (MBP), neuroserpin, lipopolysaccharide transport protein A (LptA) and tubulin.

Finally, a third line of research focuses on the ESI mechanism itself, with particular emphasis on the protein ionization mechanism and the physicochemical bases of conformational effects in protein ESI-MS. Experimental and computational methods have been employed to develop a unified model for the interpretation of protein ionization behavior based on their structural properties.

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

From channels to neuronal networks

GROUP LEADER
Becchetti Andrea
andrea.becchetti@unimib.it

RESEARCH TEAM
Meneghini S., Brusco S., Aracri P., Gullo F., Lecchi M., Pastori V.

Bioelectricity is investigated in relation to pathways and regulatory circuits of many physiological functions and information coding in neuronal systems. Knowledge about the functional regulation of the underlying electrical excitability and its role in diseases and therapeutic manipulations are of great importance in both medicine and pharmacology. Currently, increased interest is brought to the molecular level of bioelectricity as an increasing number of diseases have been shown to be related to dysfunction of ion channels or related regulatory pathways. Therefore, adequate electrophysiological methods and instrumentations are required to investigate the modulation of specific ion currents in order to understand how the local circuits work and how they integrate their signals to produce proper neuronal network activity.

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

Amyloid aggregation of polyglutamine-carrying proteins: molecular mechanisms, characterization of antiamyloid agents and toxicity in cellular models and in *Caenorhabditis elegans*

GROUP LEADER
Tortora Paolo
paolo.tortora@unimib.it

RESEARCH TEAM
Regonesi M.E., Bonanomi M., Visentin C.

Our investigations are mainly focused on ataxin-3 (AT3), a 42-kDa intracellular protein that is responsible for the polyglutamine (polyQ) disease spinocerebellar ataxia type 3 (SCA3), when the length of its polyQ tract is expanded beyond about 50 consecutive residues. Other proteins carrying expanded polyQs, such as for instance, the well-known huntingtin, share at least in part the pathogenic mechanisms with AT3. A polyQ tract expanded beyond the critical threshold results in misfolding and other structural rearrangements of the protein, which leads to aberrant interactions and the consequent formation of fibrillar amyloid-like aggregates. Final aggregation products are large fibrils, although the most toxic forms are deemed to be smaller oligomeric intermediates.
Our studies pursue the following goals:
1) Structural analysis of the aggregation intermediates. We have identified intermediate, oligomeric, SDS-soluble aggregates, which evolve into SDS-insoluble fibrils, and extensively characterized the structural features of such aggregation forms, mainly by Fourier Transform Infrared (FTIR) spectroscopy and Atomic Force Microscopy (AFM).
2) Mechanisms of toxicity of the aggregation intermediates in transgenic models. We have developed SCA3 transgenic models (*Saccharomycescerevisiae* and *Caenorahbditis elegans*) that express either wild type (Q17) or expanded AT3 (Q72 and Q130, respectively). In both organisms, we have demonstrated the toxicity of the only expanded form and provided insight into the mode by which it exerts such effects at the cellular level.
By taking advantage of investigations performed on C. elegans, we have characterized the protective mechanism(s) of different antiamyloid agents at the cellular and molecular level. To provide a comprehensive picture of the effects observed, we have complemented the in vivo investigations with those performed by physical techniques, such as FTIR and AFM.

NANOBIOTECHNOLOGIES FOR PRECLINICAL STUDIES

Fourier transform infrared spectroscopy of biological systems: from biomolecules to intact cells and tissues

GROUP LEADER
Doglia Silvia Maria
silviamaria.doglia@unimib.it

RESEARCH TEAM
Ami D. and Natalello A.

Fourier transform infrared spectroscopy (FTIR) is a label free and non-invasive technique that is successfully applied to the study of the structural properties of isolated biomolecules. In addition, this spectroscopic approach exerts an enormous attraction in biology and medicine since it enables to obtain in a rapid way a biochemical fingerprint of intact samples, such as whole cells and tissues. In our laboratory we apply FTIR spectroscopy – coupled to other biophysical approaches – to the study of protein conformational stability, misfolding and aggregation. In particular, we study the amyloid aggregation of several proteins and peptides involved in human diseases, such as β-2 microglobulin, Ataxin-3 (in collaboration with Prof. P. Tortora), and α-synuclein (in collaboration with Prof. R. Grandori).

Thanks to the use of an infrared microscope, we study protein aggregation also in situ. In particular, amyloid deposits within human tissues are under investigation. This research is carried out in the framework of the Cariplo 2014-2015 project “Structure-function relation of amyloid”. In addition, we explore the effects of different amyloid assemblies on cells, with particular interest in the response of lipid membranes.

Furthermore, by FTIR microspectroscopy supported by multivariate analysis, we study different cell processes, including cell maturation and differentiation, focusing on the role of bioactive lipids in determining the cell fate.

FTIR spectroscopy is also employed for the monitoring of processes of biotechnological relevance, including the formation of inclusion bodies within intact bacterial cells, enzymatic trans-esterification (in collaboration with Prof. M. Lotti) and fatty acid accumulation in oleaginous yeasts (in collaboration with Prof. D. Porro and Prof. P. Branduardi).

RESEARCH AREA

INDUSTRIAL AND SYNTHETIC BIOTECHNOLOGY

The aim of this thematic area of research is to reach the knowledge and consequently to develop skills and methods necessary for tailoring industrial processes based on natural and recombinant cell factories. Said activities imply the genetic manipulation of industrial strains and cell lines, the design and the construction of synthetic organisms or parts, the molecular design and the reconstruction of metabolic network. The systems and the parts so created are tested for bioconversion performances, validation of products and viability of the processes and are intended to support a sustainable and environmental friendly development of pharma-chemical and food industry a well as of biofuels and biomaterials production.

INDUSTRIAL AND SYNTHETIC BIOTECHNOLOGY

Tailoring microorganisms for the challenging conversion of biomasses into bioproducts

GROUP LEADER
Porro Danilo
danilo.porro@unimib.it

RESEARCH TEAM
Branduardi P., Bertacchi S., Berterame N. M., Brambati M., Brambilla M., Fossati T. Kuanyshev N., Marano F., Martani F., Posteri R., Signori L.

Biorefinery can be defined as the sustainable processing of biomass into a spectrum of marketable products and energy. In an economically viable biorefinery, different types of biomass feedstock (wastes are preferred) are completely converted by microorganisms to biofuels and chemicals through their metabolism. The biorefinery concept is therefore analogous to today's petroleum refinery, which produces multiple fuels and products starting from petroleum.

When exploited in bio-industrial processes, microorganisms are defined as "cell factories", whose performance, and consequently the yield and productivity of the entire process, can be influenced by the biomass and the operative conditions imposed during industrial fermentations. The choice and the genetic improvement of a cell factory, comprising its engineering to produce unnatural products, the challenge of the initial substrate and the requirement of the final product, are therefore crucial for the establishment of novel bioprocesses.

The research activities of our group are focused on different relevant aspects for the implementation of biorefinery processes, such as optimization of the raw materials exploitation, valorization of lignocellulosic biomasses and the engineering of cell factories characterized by high rate of production, productivity and improved stress resistance. In addition to *Saccharomyces cerevisiae*, widely employed for the production of several industrial products, *Zygosaccharomyces bailii*, oleaginous yeasts and basidiomyceteous fungi are exploited as cell factories for different purposes.

The ongoing lines of research are aimed to the optimization of growing conditions and to the engineering of cell factories in regard to the utilization of crude-glycerol and lignocellulose as carbon sources for biofuels production, the production of lignin- and (hemi)cellulose- modifying enzymes, the development of strains robust against weak organic acids and the production of biofuels and biochemicals.

INDUSTRIAL AND SYNTHETIC BIOTECHNOLOGY

Protein Engineering and Industrial Enzymology

GROUP LEADER
Lotti Marina
marina.lotti@unimib.it

RESEARCH TEAM
Brocca S., Sasso F., Parravicini F. (PhD student), Tedeschi G. (PhD student), Ferrari C. (undergrad.), Chmielewska S. (undergrad.), Mangiagalli M. (undergrad.)

Our group studies enzymes involved in biocatalysis, model proteins and destructured proteins to highlight the molecular basis of stability, specificity and interactions and, when appropriate, to improve these properties. The technical approach is based on protein engineering, biochemical and biophysical analysis. In the following we provide some highlights about the major issues of our research.

In the field of industrial biotechnology, microbial lipases employed in biodiesel production are investigated as for their robustness toward organic solvents used in the transesterification reaction. We have compared mathematical models and experimental reaction kinetics and found that different mechanisms account for lipase inactivation.

A dimeric bacterial protease (APH) is our model to study the interactions between intramolecular domains and between subunits of a quaternary structure. We cloned and produced APH as a recombinant protein. The X-ray structure of APH shows domain swapping between subunits, a mechanism at the basis of both physiological oligomerization and dysfunctional aggregation. We target APH with site-directed mutagenesis to investigate how domain association affects overall stability, catalytic efficiency and substrate specificity.

High flexibility and the lack of a defined 3D structure in the absence of a partner protein are the main traits of Intrinsically Destructured Proteins (IDPs). Among IDPs, we investigate the properties of Sic1 and Whi5, involved in the cell cycle of the yeast Saccharomyces cerevisiae. Presently, we are studying the conformational effects of the association of Sic1 with its interaction partners Clb5 and Cdk1, which have defined 3D structures. IDPs can also work as “entropic bristles”, molecular devices able to avoid protein aggregation. Indeed, an aggregation–prone protein fused with an IDP can escape aggregation. Which are the general rules an IDP must fulfill to exhibit anti-aggregation properties? Different IDPs must be tailored to match the features of different globular proteins? We are working to answer these questions

INDUSTRIAL AND SYNTHETIC BIOTECHNOLOGY

Computational Methods in Industrial Biotechnology and their application to energy and health issues

GROUP LEADER
De Gioia Luca

luca.degioia@unimib.it

RESEARCH TEAM
Zampella G., Bertini L., Lambrughi M., Filippi G., Arrigoni F., Prosdocimi T., Nicotra S., Tunesi A., Barbanotti L., Piccirillo M.

Computational methods applied to biotechnology fields can provide a key to disclose molecular events that underlie properties and features related to biosystems: for instance structure function relationship in proteins can in principle be disclosed. Different techniques are employed to elucidate molecular mechanisms underlying bio-catalysis, protein dynamics and protein-protein interactions. Ab-initio methods (such as DFT) allow to investigate the chemistry of enzyme cofactors and simultaneously help design new synthetic molecules inspired to living systems. Along this research line, we move in different directions.

i) In the first one we focus on hydrogenases, enzymes extremely relevant in the renewable energy field for their high rate of H2 production. Hydrogen can be considered a completely forward looking energy vector (fuel) being it simultaneously clean (its combustion is CO2-free, producing nothing but water) and high-density. The study of these enzymes has mainly led to the discovery of which stereo-electronic features are needed for an efficient H2 activation/production: this is the first step to conceive new biomimetics able to simulate the high activity of related biosystem.

ii) Also related to energy issues are investigations on biosystems (formate dehydrogenase) and synthetic iron-based compounds (low cost), able to incorporate H2 into CO2 chemically by affording high added value chemicals, such as formic acid. This strategy allows to store H2 reversibly in a more easily transportable form and also to use an abundant and thus extremely cheap feedstock, such as CO2.

iii) Studying the correlation between Alzheimer's disease and oxidative stress, we explored the interactions of copper ion with amyloid β peptide, in order to disclose the nature of the interaction and consequently how the production of ROS may occur.

Another research line consists in improving the protocol based on detection of carbohydrate-deficient transferrin for diagnosis of chronic alcohol abuse using both molecular dynamics, docking and experimental approaches.

RESEARCH AREA

SYSTEMS BIOLOGY

In a systems biology approach, biological processes are taken to be the results of complex, coordinated, dynamic, non-linear interactions of a large number of components, which are shaped by time and space constrains. These dynamic interactions generate, as emergent property of the system, the corresponding function, that therefore is not found in individual components, but only in their networking and must be studied through the integration of biomolecular analysis (including omics) and mathematical modeling. The area accommodates different types of skills (biochemical, molecular biological, genetic, chemical, computational, etc.) and addresses relevant cellular functions (growth and cell cycle, differentiation, cell death, signal transduction) in model organisms of different evolutionary complexity.

SYSTEMS BIOLOGY

NMR applications to the molecular characterization of biosystems

GROUP LEADER
Nicotra Francesco
francesco.nicotra@unimib.it

RESEARCH TEAM
Airoldi C. and Guzzi C.

The group is devoted to the application of NMR spectroscopy to the study of ligand-receptor interactions of biological and biomedical relevance. The main target is represented by amyloid peptides / proteins (Aß peptides, Prp106-126, ataxin-3 and their aggregates), while putative ligands are both synthetic compounds and the components of more complex organic matrices, in particular natural extracts.

At the same time, the group is developing new methods allowing to carry out ligand-receptor interaction studies in heterogeneous systems, i.e. systems in which at least one of the actors of the binding is not soluble in physiological conditions. The final goal are new methods suitable for carrying out molecular recognition studies involving molecular entities anchored to the surface of cells (such as receptors or membrane components), resins and insoluble materials, nanostructures and even fragments of tissues.

NMR spectroscopy also allows to identify and quantify all compounds present in a complex matrix with a concentration above approximately 1 microM. Thus NMR can be exploited for the metabolic profiling of biological samples such as culture media, cell extracts, biological fluids, with the goal of identifying biomarkers characteristic of some human diseases. Our group is particularly concerned with the application of these methodologies to study the metabolism of cancer cells and budding yeast and to characterize biofluids from patients with lung diseases.

SYSTEMS BIOLOGY

Systems Biology approaches to cell proliferation in eukaryotes

GROUP LEADER
Vanoni Marco
marco.vanoni@unimib.it

RESEARCH TEAM
Busti S., De Sanctis G., Sacco E., Spinelli M., Trinchieri M., Vergani V.

Systems Biology holds that complex cellular functions are generated as system-level properties endowed with robustness, each involving large networks of molecular determinants, generally identified by "omics" analyses.
We are interested in understanding the coordination between cell growth, cell metabolism and cell cycle in both lower (yeast) and higher eukaryotes (normal and transformed mammalian cells).
The role the played by the sensing of the carbon source has been addressed by biochemical experiments that indicate that glucose sensing is playing an important role in affecting the yeast cell size. The ability of different nitrogen source in promoting yeast cell growth is also studied.
In calcium-depleted cells growing in media supplemented with good fermentable carbon source, inefficient energetic metabolism in the absence of a switch to respiration is unable to to sustain high energetic demands leading to slow growth, oxidative stress and cell death of a sizable fraction of the cell population. This altered phenotype can be rescued by forcing respiration or decreasing protein synthesis, thereby indicating that the balance between the efficiency of the carbon source metabolism and anabolic requirements plays a key role in cell fate.
In yeast the connection between metabolism, cell growth and cell cycle is being explored by a multi-scale modeling approach which makes use of models of different level of granularity (in collaboration with CNR-IASI in Rome). In particular, we showed that multiple phosphorylation of a transcriptional inhibitor of the G1/S transcription acts at the core of a consensus model for the G1/S transition.
Using steady-state constrained models (in collaboration with the DiSCo Department in Milano-Bicocca) coupled to metabolic investigations, we are studying the molecular bases of the enhanced growth phenotype characteristic of many tumor cells.

SYSTEMS BIOLOGY

Snf1/AMPK regulates a tumor-like rewiring of metabolism in *Saccharomyces cerevisiae*

GROUP LEADER
Cocetti Paola
paola.cocetti@unimib.it

RESEARCH TEAM
Nicastro R., Tripodi F., Reghellin V.

Snf1 is a highly conserved Ser/Thr kinase required in yeast to adapt to nutrient limitation and to respond to stress conditions (Hedbacker and Carlson, 2008). We showed that Snf1 is also involved in the regulation of the cell cycle under glucose repressed conditions (Busnelli et al., 2013; Pessina et al., 2010). Recent studies evidenced that the activation of AMPK is involved in growth control and tumorigenesis and that ablation of the catalytic subunit AMPKα1 favors glucose uptake and aerobic glycolysis to support proliferation of cancer cells (Faubert et al., 2014).
By integrating multiple techniques (in collaboration with J. Nielsen Chalmers University of Technology, Göteborg, Sweden and C. Airoldi University Milano Bicocca), we investigated the parallelism between AMPK deficient mammalian cells and snf1Δ yeast cells, highlighting the similarities and describing the rewiring of yeast metabolism occurring upon Snf1 loss. We show that lack of Snf1 causes a large transcriptional deregulation in 2% glucose, but not in 5% glucose. snf1Δ growing in 2% glucose show increased glycolysis and fatty acids overproduction, fueling of carbon into the TCA cycle and glucose-dependent accumulation of glutamate. These findings provide the usefulness of yeast as a model organism for the study of cancer metabolism and will allow to simplify the study of metabolism plasticity arising after driver changes in signaling pathways.

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SYSTEMS BIOLOGY

Mechanisms of Neurodegeneration mediated by glial cells and Neuroprotection by Nerve Growth Factor (NGF)

GROUP LEADER
Colangelo Anna Maria

annamaria.colangelo@unimib.it

RESEARCH TEAM
Ippolito V.M. (1,3), Formenti M. (1), Riganti V. (1), Moscariello P. (1), Papa M. (2,3), Alberghina L. (3,4) and Colangelo A. M.(1,3,4).

1) Lab. of Neuroscience "R. Levi-Montalcini", Dept. of Biotechnology and Biosciences, University of Milano-Bicocca;
2) Lab. of Morphology of Neuronal Network, Dept. of Public Medicine, Second University of Napoli;
3) SYSBIO, Centre of Systems Biology, University of Milano-Bicocca;
4) NeuroMI Milan Center for Neuroscience, University of Milano-Bicocca.

Glial cells play a crucial role in neurodegenerative disorders by determining the onset of chronic neuropathological processes. To elucidate molecular mechanisms of glial activation in the disruption of neuro-glial network homeostasis, we are using an in-vitro model of reactive gliosis based on pure populations of astrocyte cells treated with the pro-inflammatory cytokine Tumor Necrosis Factor-a (TNF-α) or mixed astroglial cells treated with lipopolisaccaride (LPS). We observed that both inflammatory mediators increased glial cell proliferation, as determined by cell counts at specific time-points, as well as by BrdU incorporation, without affecting astrocytic survival. This process was strongly reduced by co-treatment with Nerve Growth Factor (NGF), a neurotrophin previously shown to have anti-gliosis activity in animal models of peripheral nerve injury (Colangelo et al., 2008, Cirillo et al. 2010 and 2011).
Interestingly, glial proliferation was paralleled by: 1) increased methalloproteinases activity (MMP-6 and MMP-9), as determined by zymography of conditioned media (CM) from cytokine-activated astrocytes, 2) alteration of NGF/proNGF levels and 3) modification of synaptic components (glutamate/GABA systems) (Colangelo et al., 2008, Cirillo et al. 2010 and 2011). In addition, glial activation strongly affected NGF receptors levels by inducing reduction of TrkA and increase of p75. All these changes were partially restored by NGF treatments.
Besides its effect on glial cells, NGF was found to possess neuroprotective activity on primary cortical neurons exposed to conditioned media (CM) from activated astrocytes by improving their survival through mechanisms involving regulation of mitochondrial function and modification of genes specifically involved in synaptic plasticity.
Overall these data will help to construct a concept map of reactive gliosis to better define glial-mediated mechanisms of neurodegeneration and the role of NGF in neuroprotection.

SYSTEMS BIOLOGY

Glucose metabolism as a tool for discovering cancer therapeutic targets

GROUP LEADER
Chiaradonna Ferdinando
ferdinando.chiaradonna@unimib.it

RESEARCH TEAM
Claudia C. (FIRB Researcher), H. De Vitto (Post-doc), Roberta P. (Post-doc), Ricciardiello F. (PhD student), Votta G. (Post-doc)

Glucose is involved in different metabolic pathways essential for cancer proliferation, survival and migration. Indeed, a possible anticancer therapeutic approach is to target glucose metabolism. In this regard, phenotypical and molecular analyses of cancer cells under glucose deprived condition can be an useful strategy to unmask mechanisms involved in cancer growth. Exploiting this approach, we have discovered different mechanisms essential to glucose-addicted cancer cells. For instance, the inhibition of the Hexosamine Biosynthetic Pathway leads to Unfolded Protein Response and cancer cell death. Hence, we are collaborating with other groups of the department to produce specific modulators of HBP to deeper investigate its role in cancer cells.
While the most part of cancer cells die in glucose deprivation, some of them survive and increase their aggressiveness. Therefore we are searching possible mechanisms leading to this “resistant” phenotype, whose discovering could be useful to develop new therapeutic strategies complementing the used ones. In this regard, on the basis of our previous published data identifying a significant deregulation of cAMP/PKA pathway in cancer cells, we are deeply investigating the role of this pathway in glucose deprived cancer cells. Since it regulates several cellular processes, we are currently performing different analyses (phenotypical, functional, molecular) by using also different “omic” approaches associate to bioinformatics analysis. Our data indicate that cAMP/PKA pathway may be an essential peacemaker to permit cancer cell survival under stress condition. The role of the pathway in cancer aggressiveness is currently investigated.
Finally, we are collecting and integrating our experimental data about cancer cells in glucose deprivation to build a computational model as possible tool to discover novel targets favoring cancer cell death bypassing resistance mechanisms.

*Internal Collaboration:*B. La Ferla, C. Airoldi, D. Besozzi, G. Mauri

RESEARCH AREA

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

This research area includes 10 research groups having a common interest in the study of molecular and genetic mechanisms of normal and pathological cellular functions. These basic science studies, that are in several cases carried out in collaboration with researchers active in experimental medicine, represent a starting point towards the exploration of mechanisms underlying important human pathologies, such as cancer, hereditary diseases, neurodegeneration and senescence, and bacterial infection. These groups are highly competent in several research areas, and their methods and approaches are widely shared, favoring important cultural and experimental interactions (cellular and animal models, genomics, proteomics, stem cells, genetics of eukaryotic and prokaryotic microorganisms).

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

Roles of the Sox2 transcription factor in brain development and disease, and in neural stem cells

GROUP LEADER
Nicolis Silvia K.
silvia.nicolis@unimib.it

RESEARCH TEAM
Favaro R. (Post-doc), Mercurio S. (Post-doc),
Bertolini J. (PhD student), Pagin M. (PhD student),
Barone C. (undergrad.), Bottes S. (undergrad.), Gesuita L. (undergrad.)

Our laboratory is interested in how genes orchestrate brain development, and, by their disfunction, can cause brain disease. We also investigate the genetic control of neural stem cells (NSC), whose balanced self-renewal and differentiation shapes the brain in embryogenesis, and allows, at some locations (e.g. hippocampus), neurogenesis in the adult brain. We focus on the transcription factor Sox2, encoded by a gene whose mutation causes a spectrum of brain abnormalities in humans. Sox2 expression marks the developing central nervous system from the earliest stages, and therein, NSC. We address Sox2 function by conditional mutagenesis in mouse: by a “Sox2flox” mutation, we delete Sox2 in specific brain regions and developmental stages. Sox2 is required for the maintenance of NSC in vivo (postnatal hippocampus) and in culture, and in the developing ventral telencephalon. We now focus on novel functions of Sox2 that we identified in early hippocampus, in the determinationof cerebral size (Sox2 mutants develop microcephaly), in cerebral cortex arealization, and in the cerebellum (where Sox2 loss causes ataxia). We address Sox2 target genes: we identified Shh and Nkx2.1 (encoding a cytokine, and a transcription factor) as mediators of Sox2 function in hippocampus and ventral forebrain development. By genomic approaches, we now identified Sox2 targets in brain-derived NSC (ChIPseq; RNAseq), and long-range interactions in NSC chromatin (ChIA-PET), linking genes to distant regulatory elements, that depend on Sox2. These experiments identify novel potential effectors of Sox2 function in brain disease, and suggest that Sox2 may affect gene regulation by the maintenance of specific long-range interactions in NSC chromatin. By our Sox2flox mutation, we also demonstrated a requirement for Sox2 in the maintenance of cancer stem cells (CSC) in a mouse glioma; genes differentially expressed following Sox2 loss are now providing testable hypotheses as to genetic pathways mediating Sox2 roles in CSC maintenance, that may provide objectives for therapeutic intervention.

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

Molecular Biology Lab focused on RNA processing and its implications in human diseases

GROUP LEADER
Barabino Silvia
silvia.barabino@unimib.it

RESEARCH TEAM
Lenzken S.C.(PostDoc), Alvarez R. (Doctor), Loffreda A. (PostDoc), Rigamonti A. (PhD student), Filosa G. (PhD student)

Precursor mRNAs are synthesized from DNA in the nucleus and co-transcriptionally processed to generate mature transcripts by capping at the 5' end, splicing of introns and 3' end polyadenylation. These mature mRNAs are exported to the cytoplasm where they serve as templates for protein synthesis. The specific translation rate of each transcript is governed by several post-transcriptional mechanisms, i.e. mRNA transport, RNA quality-control, storage and degradation. These processes are tightly regulated and it is becoming clear that RNA metabolism dysfunctions are strongly linked to human diseases.

Our lab is interested in the study of some aspects of mRNA metabolism and its implications in neurodegeneration (ND). Current research projects are the study of: 1) alternative splicing (AS): we have noticed that Brahma (BRM), a subunit of the SWI/SNF complex, is able to control the choice of some Alternative Last Exons (ALE). BRM recruiting the BARD1/BRCA1 complex causes a preferential choice of the proximal ALE. Indeed we have identified a short BRM transcript encoding a small protein: Brahma short (BrmS). Its function is yet unknown, but its protein structure suggests a possible role as dominant-negative protein; 2) microRNAs mediated post-transcriptional regulation: we study the miRNA expression pattern in Amyotrophic Lateral Sclerosis (ALS) models in order to clarify their putative impact on the disease. We identified a small group of up-regulated miRNAs in cells and mice models of ALS as well as in sporadic ALS patients. Currently, we are studying their targets; 3) the RNA-binding protein FUS, associated to ALS pathology. This protein has been involved in RNA processing and in the DNA Damage Response (DDR). We are studying the effects of its depletion at both gene expression and AS levels. Furthermore we are characterizing some relevant FUS interactions and studying their relevance in the DDR.

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

Yeast as a model system to study human aging

GROUP LEADER
Vai Marina
marina.vai@unimib.it

RESEARCH TEAM
Dr. Orlandi I., Dr. Coppola D.P., Dr. Ronzulli R., Dr. Strippoli M.

Cellular longevity is known to be strongly influenced by both carbon sources and the meticulous control of metabolite fluxes among cellular compartments. These phenomena are evolutionary conserved from single-celled organisms such as yeast to complex multicellular eukaryotes and have together provided valuable information in order to comprehend mechanisms underlying aging and promote therapeutic interventions. In this context, the yeast chronological lifespan (CLS) paradigm offers the opportunity to study the aging process of postmitotic quiescent mammalian cells and how metabolic intermediates are involved in the regulation of longevity.
We are currently focusing on:
- How key intermediates of carbon metabolism such as pyruvate, the end-product of glycolysis, or acetate, precursor of acetyl-CoA, influence through their faith the different metabolic changes which characterize chronological aging;
- How metabolism in different cellular compartments, such as energy metabolism in mitochondria and lipid accumulation/β-oxidation in peroxisomes, is related to main cellular events like respiration, ROS production, nutrient storage and autophagy;
- How the NAD+-dependent deacetylase Sir2, the founding member of the Sirtuins family, acting as a metabolic sensor modulates cell metabolism during chronological aging.
Elucidation of such molecular/physiological aspects involved in aging will provide an improvement in the development of therapeutic approaches aimed at improving the quality of life.

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

Role of transcription factors in the regulation of hemoglobin switching

GROUP LEADER
Ronchi Antonella
antonella.ronchi@unimib.it

RESEARCH TEAM
Fugazza C. (Post-doc), Elangovan S. (Post-doc), Barbarani G. (PhD student), Font Monclùs I. (PhD student), Cova G. (undergrad.), Annibaldis G. (grad.)

Red blood cells (RBCs) are essential for life in vertebrates due to their function in oxygen delivery. During erythroid differentiation and maturation, the production of α- and β-globin chains in the right ratio is critical to form stable adult hemoglobin. Imbalance or alterations of these components due to mutations in globin genes and/or their regulators can be deleterious for RBCs and their precursors and may lead to thalassemias and sickle cell disease. Clinical evidence indicates that increasing levels of fetal hemoglobin can ameliorate the severity of these diseases. Indeed patients with β- thalassemia that co-inherit HPFH (Hereditary Persistence of Fetal Hemoglobin) generally have a milder clinical course. Therefore, one of the promising strategies to cure hemoglobinopathies is by "reactivating the fetalhemoglobin".

The aim of our group is to identify the network of genetic and molecular interactions that are important for the γ to β globin switch and for the repression of γ globin in adulthood. Since the mechanism of globin switching is conserved among mammals, we performed gene expression profiling of purified fetal liver cells, isolated at different times during murine gestation, to cover the period when the switch from fetal to adult hemoglobin occurs. These data revealed new key regulators of the hemoglobin switching and factors important during differentiation. Among these, SOX6, COUP-TFII, BCL11a are the most notable, and has been proposed as candidate modulators of γ-globin expression. To have a better insight on their role, we decided to manipulate their expression and to validate their functional role in the regulation of the γ to β globin switch.

Overall, our study may help to elucidate part of the network of functional and physical interactions between several transcription factors, responsible for the negative regulation of γ globin gene in adulthood.

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

Structural and functional characterization of proteins

GROUP LEADER
Fusi Paola
paola.fusi@unimib.it

RESEARCH TEAM
Forcella M.

Role of sialidase NEU3 in colorectal carcinogenesis
Colorectal cancer (CRC) is the second leading cause of cancer-related death in the Western countries. Newer therapeutic options include targeted therapies, such as EGFR antagonists. Genetic alterations of EGFR and its downstream signaling effectors may predict the efficacy of these drugs. The human plasma membrane sialidase NEU3 interacts with EGFR. In our laboratory, in collaboration with Dr. Frattini (Istituto Cantonale di Patologia, Locarno) a correlation between EGFR and NEU3 deregulation has been shown, in CRC patients as well as in cell lines. The activation of EGFR downstream pathways has been demonstrated in CRC cells overexpressing NEU3.

Trehalase from C. riparius as a target for bioinsecticides
Trehalase inhibitors have a great potential as human safe bioinsecticides. Recombinant trehalase from the Diptera C. riparius has been expressed in E. coli and is now being tested as a target for new synthetic bioinsecticides, in collaboration with Prof. Parenti and Prof. Cipolla (Universiy of Milan-Bicocca), as well as Dr. Cardona (University of Florence). Some imminosugars have been shown to selectively inhibit C. riparius trehalase and are being tested in vivo in S. littoralis, in collaboration with Prof. Casartelli (University of Milan).

Biochemical characterization of tumor foci
The prediction of carcinogenic potential for humans relies on the extremely costly bioassay in animals (OECD, TG451). In vitro cell transformation assays (CTAs) closely mimick some stages of the in vivo carcinogenesis process and have the potential to detect both genotoxic and non genotoxic carcinogens. In collaboration with Dr. Urani (University of Milan-Bicocca), we are working at the development of a quantitative method for foci scoring and classification, based on the identification of biochemical markers of cell transformation and proliferation, following exposition to cadmium.

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

Mechanisms preserving genome stability in *Saccharomyces cerevisiae*

GROUP LEADER
Longhese Maria Pia
mariapia.longhese@unimib.it

RESEARCH TEAM
Clerici M. (RU), Bonetti D. (Post-doc), Manfrini N. (Post-doc), Trovesi C. (Post-doc), Martina M. (Post-doc), Cassani C. (Post-doc), Gobbini E. (PhD student), Cesena D. (PhD student), Villa M. (PhD student), Colombo C.V. (undergrad.), Menin L. (undergrad.), Gnugnoli M. (undergrad.), Rizzo E. (undergrad.)

The genome of living cells is continuously exposed to endogenous and exogenous agents that induce a large spectrum of DNA lesions, whose proper and efficient removal is essential to maintain genome stability. To face DNA damage, cells have evolved a powerful DNA Damage Response (DDR), which is a network of proteins capable of detecting DNA lesions and signaling their presence to activate pathways that repair DNA lesions, delay cell cycle progression, or eventually eliminate genetically unstable cells by inducing cell death. The DDR is critical to prevent tumorigenesis, as indicated by the cancer-prone phenotype of several DDR syndromes. Furthermore, somatic mutations in DDR genes have been found in several cancer types.

Among the many types of DNA damage, the DNA double strand break (DSB) is the most severe one because it has the potential to cause mutations, chromosomal rearrangements and loss of genetic information. Interestingly, the natural ends of linear eukaryotic chromosomes resemble DSBs. But, while randomly-occuring DNA breaks are potent stimulators of the DDR, the natural ends of linear chromosomes are packaged into protective structures that suppress DNA repair/recombination activities. This protective function, referred to as “telomere capping”, depends on the presence of telomere-associated proteins and species-specific telomeric DNA repeats. The presence of a proper telomere structure is extremely important to preserve genomic stability and prevent tumorigenesis.

Our research activity aims to elucidate the molecular mechanisms underlying the DDR, with a particular focus on the response to DSBs and on telomere maintenance. As many aspects of the DDR and telomeres are remarkably conserved throughout evolution, the organism chosen to tackle these issues is yeast *Saccharomyces cerevisiae*, which is widely recognized by the scientific community as a powerful model system to study the cellular response to DNA damage.

GENETIC AND MOLECULAR MECHANISMS IN CELLULAR BIOLOGY AND PATHOLOGY: IMPLICATIONS FOR HUMAN DISEASE

Molecular genetics of bacteria

GROUP LEADER
Polissi Alessandra
alessandra.polissi@unimib.it

RESEARCH TEAM
Sperandeo P. (PhD Fellow), Martorana A.M. (PhD Fellow), MaccagniE.A. (Graduate Fellow), Morè N. (PhD Student), Boscaini S. (Undergrad.)

The Gram-negative bacteria cell envelope consists of three layers: an inner cytoplasmic membrane (IM) made of phospholipids, an outer asymmetric membrane (OM) containing phospholipids in the inner leaflet and a unique glycolipid lipopolysaccharide (LPS) in the outer leaflet, and a thin layer of peptidoglycan (PG) sandwiched between them.

Our Lab has long been implicated in the study of OM biogenesis. In particular our studies contributed to the discovery of the Lpt protein machinery responsible for LPS transport to the OM in *Escherichia coli*. This essential machinery is composed by seven proteins (LptABCDEFG) located in each envelope compartment and forms a transenvelope complex that operates as a single device. Alongside of the study of molecular mechanism for LPS transport, our Lab has recently started the characterization of factors implicated in envelope biogenesis/homeostasis that are virulence factors likely to play a key role in host-pathogen interaction, such as AsmA and VacJ proteins (in collaboration with Dr.Typas at EMBL in Hidelberg and Dr.Mauri at CNR in Milan). The major goal of this project is to understand the molecular role of AsmA and VacJ in envelope biogenesis and during infections, also exploiting ex vivo and in vivo model systems (in collaboration with prof. Bernardini at Sapienza University in Rome and prof. Rescigno at IFOM-IEO in Milan). Based on current data we believe that these factors are ideal candidates to explore cross talk between the host and the pathogen.

Furthermore, we focused our attention on the strategies adopted by E. coli to coordinate LPS biogenesis with other cell envelope components. In particular we found that block of LPS transport modulates the level of proteins involved in PG synthesis and remodelling. We are currently investigating how these changes in PG structure leads to rescue the OM stability (in collaboration with prof. Vollmer at Newcastle University).

RESEARCH AREA

BIODIVERSITY, MARINE SCIENCE, AND FOOD RESEARCH

The research topics in the scientific area are committed to the study of the processes affecting and threatening the biodiversity in natural and agricultural ecosystems. The ultimate goal is to improve the quality of life and health of living beings. The most recent genomic techniques and tools for the molecular identification are integrated with traditional approaches in the study of biodiversity, marine sciences and food safety. The researches move from a modern view of biodiversity, that is a common universal good but also a source of useful resources to be managed to preserve natural ecosystems and human activities. The XXI century biodiversity implements research, management and applied science to face the current huge global challenges.

BIODIVERSITY, MARINE SCIENCE AND FOOD RESEARCH

Study of Birds Migration and Ecology of Glacial Environments

GROUP LEADER
Ambrosini Roberto
roberto.ambrosini@unimib.it

RESEARCH TEAM
Sicurella B. (PhD student), Musitelli F. (PhD student)

Our research group is composed by Dr. Roberto Ambrosini and two PhD students, Beatrice Sicurella and Federica Musitelli. The group's interests mainly focus on Ecology and Conservation Biology, covering a broad field of applied and theoretical topics. We are currently following two main lines of research. The first line is about migratory birds. We are studying them with two different approaches. First, we are using birds ringing data to study migratory behaviour of four target species of passerines: Barn Swallow (Hirundo rustica), Common Blackbird (Turdus merula), Song Thrush (Turdus philomelos) and European Robin (Erithacus rubecula). More in detail, we are investigating migratory flows and migratory routes of these species adopting a statistical and computational approach. We aim at identify possible effects of climate change on timing of migration and on shifts in migratory flyways and wintering and breeding quarters. Second, we are partner of an international project on the study of bird migration, both at population and individual level, by means of miniaturized instruments, the light-level geolocators. In the past years we applied hundreds of geolocators on Barn Swallows and Common Swifts (Apus apus) and we are now analysing the data collected from these devices. These instruments allowed an accurate identification of the wintering areas of Barn Swallows breeding in Northern Italy. The second research topic of our group is the ecology of glacial environments of Alps, Himalaya and Karakoram. Glacial environments are the largest freshwater environment on Earth, but their ecological investigation is still in its infancy. We are collaborating with microbiologists and glaciologists to investigate the ecology of peculiar supraglacial environments, like cryoconite holes and supraglacial debris. We observed an increase in the complexity of bacterial communities in the debris that covers debris-covered glaciers, toward the glacier terminus.

BIODIVERSITY, MARINE SCIENCE AND FOOD RESEARCH

GLYCO-NANOTECNOLOGY: Glyconanoparticles and Glyco-Molecular tools for targeting and as modulators-inhibitors-drugs. New devices for molecular interactions studies from biosensors to bio-functionalized NMR tools. Bacterial microencapsulation for functional food production.

GROUP LEADER
La Ferla Barbara
barbara.laferla@unimib.it

RESEARCH TEAM
D'Orazio G. (Post-doc), Brighetti D. (undergrad.), Moretti F. (undergrad.), Munizza L. (undergrad.)

Glyconanoparticles (G-NPs) constitute versatile nanoplatforms for drug delivery and targeting. We focus on two typologies of NPs: 1) glycol-based NPs and 2) glycol-decorated NPs. The first type are cellulose nanocrystals (CNCs). Despite there are examples of drug loaded CNCs in the literature, there is no study of in-vivo pharmacokinetic study of such NPs. We are addressing this topic and preliminary data show an important tropism towards bone tissue, promising fact for the treatment of bone related pathologies. We are also exploring glycol-decorated NPs, in particular biodegradable polymer-based NPs, decorated with glycoderivatives for the targeting of the Blood Brain Barrier, a key feature for the treatment of important neurodegenerative pathologies such as Alzheimer's disease.

Glyco-Molecular tools: in this context we are developing selective inhibitors/modulators of the Hexosamine Biosynthetic Pathway, as potential antitumoral drugs and as chemical tools for the treatment of related pathologies.

Biosensors: The selective detection of biomolecules is a key feature for the development of novel and high-throughput diagnostic tools. We are developing conductive polymer based biosensors in which the polymer has been selectively functionalized with glyco-derivatives and used for the detection of lectin proteins via an electrochemical analysis of polymer conductivity.

Bio-functionalized NMR tools: this work has the aim of functionalizing the inner surface of an NMR tube in order to covalently support biomolecules for STD-NMR interaction studies. This immobilization could allow the recycling of biomolecules, such as proteins thus overcoming limitations due to scarce availability.

Collaborations:*UNIMIB* Prof. Marco Orlandi/ Dr. Luca Zoia, Dr. Giulio Sancini, Dr.ssa Cristina Airoldi, Dr. Ferdinando Chiaradonna, Prof. Luca Beverina, Dr.ssa Patrizia Di Gennaro, *CNR* Dr.ssa Barbara Vercelli, *MARIO NEGRI INSTITUTE* Dr. Mario Salmona/ Dr. Paolo Bigini

BIODIVERSITY, MARINE SCIENCE AND FOOD RESEARCH

Applied and Environmental Microbiology Group

GROUP LEADER
Di Gennaro Patrizia
patrizia.digennaro@unimib.it

RESEARCH TEAM
Zampolli J., Di Canito A., Presti I., Boccarusso M., Colombo E.

Bacteria belonging to the Pseudomonas and Rhodococcus genus involved in the metabolism of xenobiotic compounds
The widespread environmental contamination has led to improve studies on the metabolism of xenobiotic compounds either in Gram-negative as Pseudomonas, and in Gram-positive bacteria as Rhodococcus. In this perspective, our research is focused on the isolation and identification of genes and catabolic pathways of different xenobiotic compounds in these bacteria. Sequence analysis of genomic regions allowed to identify different gene clusters containing genes for naphthalene, o-xylene and n-alkanes catabolism. Genetic studies on bacteria belonging to Rhodococcus genus are of particular interest because these widespread microorganisms exhibit a wide range of metabolic properties that can leads to an increasing of the environmental soil quality.

Study of microbial communities in water and soils
An example is the microbial characterization of soil community from energy crops plants. Energy crops are non-food plants used to make biofuels. Perennial energy crops are considered more suitable because have positive effects on soil carbon sink function, a soil property that enhance the carbon sequestration from the atmosphere through crop residues. The aim of the research is to determine the impact of biomass crop cultivation on soil quality and microbial community structure in a South European agricultural area.

Probiotic bacteria and the effect of microencapsulation on their viability
Probiotic bacteria, are known to help in balancing the intestinal microbiota, resulting in enhanced overall health, wellbeing, and boosts immune system. New probiotic strains were identified and characterized for their ability to promote the human well-being. The presence and transferability of antibiotic resistance genes and the ability of the new isolates to inhibit the growth of pathogens typical of the gastrointestinal tract was investigated. The entrapment of bacteria in a matrix of polymers was investigated to enhance the positive effects of probiotics.

RESEARCH AREA

TARGET IDENTIFICATION AND DRUG DEVELOPMENT

The Area assembles the know-how and technological platforms for the study of cellular mechanisms of disease, identification/validation of therapeutic targets and their exploitation in the design and synthesis of new candidate molecules for diagnostic and therapeutic development. The approach is based on the confluence of expertise in organic and pharmaceutical chemistry, cell physiology and pharmacology. Synergy between such components covers the best part of preclinical drug development.

TARGET IDENTIFICATION AND DRUG DEVELOPMENT

Cardiac cell physiology: mechanisms of arrhythmias from human to in silico models

GROUP LEADER
Zaza Antonio
antonio.zaza@unimib.it

RESEARCH TEAM
Rocchetti M. (RU), Altomare C. (RTD), Mostacciuolo G. (PTA),
Sala L. (PhD student), Ronchi C. (PhD student), Bernardi J. (PhD student)

Long QT Syndrome modelled with human induced pluripotent stem cells (hiPSC)
The Long QT Syndrome (LQTS) is an arrhythmogenic disorder characterized by a prolongation of QT interval. Its cardiac phenotype can be recapitulated in vitro by human induced pluripotent stem cells (hiPSC)-derived cardiomyocytes (CM). Thanks to collaborations with M. Gnecchi (University of Pavia) and A. Moretti (University of Munich) groups, we characterized hiPSC-CM to assess the underlying mechanisms of 1) A341V-KCNQ1-LQT1 asymptomatic vs symptomatic phenotype, 2) QT prolongation associated to CALM1 mutation. Both studies are funded by PRIN 2010BWY8E9.

Effects of INaL inhibition in a model of acute ischemia
Reported beneficial effects of the late sodium current (INaL) blockade during global ischemia suggest intracellular Na+ (and Ca2+) overload at the cellular level. To test whether INaL involvement contributes to ischemic damage, isolated cardiac myocytes were exposed to an ischemia-simulating solution. The effect of the INaL blocker ranolazine was evaluated on cell shortening, electrical activity and intracellular ion dynamics by video-edge, patch-clamp and epifluorescence techniques respectively. The study is funded by Gilead Sciences (Fremont, CA).

IKr impact on repolarization and its variability assessed by Dynamic-Clamp
Abnormalities of the rapid component of the delayed rectifier K+ current (IKr) are associated with prolongation and instability of cardiac repolarization. However, the impact of a given channel abnormality on electrical activity remains difficult to predict. Dynamic-Clamp (DC) is a promising approach, because it allows to test how the properties of a numerically modelled current (mIKr) affect the action potential (AP) generated by a myocyte. This work exploits DC to systematically analyze the effect of changes in IKr conductance and gating properties on guinea-pig AP duration and its time-variability, an index of electrical instability. The study is funded by FAR 2013.

TARGET IDENTIFICATION AND DRUG DEVELOPMENT

Synthetic and natural Toll-like Receptor 4 modulators: a new generation of therapeutics

GROUP LEADER
Peri Francesco
francesco.peri@unimib.it

RESEARCH TEAM
Calabrese V.(Post-doc), Cighetti R. (Post-doc),
Minotti A. (PhD student), Ciaramelli C. (PhD student),
Sestito S. E. (PhD student), Tacchini M. (undergrad.), Perrone A. (undergrad.)

Toll-like receptor 4 (TLR4) detects minute amount of Pathogen Associated Molecular Patterns, PAMPs, namely bacterial endotoxins and activate the immune and inflammatory responses to pathogen infections. However, deregulated or excessively potent TLR4 response to bacterial endotoxins or endogenous ligands generates infectious, inflammatory and autoimmune syndromes that still lack pharmacological treatment. Our research is focused on the study of TLR4 role in different pathologies and on the rational design and synthesis of small-molecule TLR4 modulators. In last ten years new molecules (mainly glycolipids) capable to interact with innate immunity receptors MD-2 and CD14, thus interfering with the molecular processes of TLR4 activation and signaling, were developed in our lab. Several generation of TLR4 antagonists were patented and some of these compounds (named IAXO molecules) are now commercially available as selective TLR4 blockers. These compounds have been successfully used by several research group to dissect the extracellular TLR4 activation process by chemically silentiating CD14 and/or MD-2 receptors. The high specificity and efficiency of some of synthetic glycolipids, and their very low toxicity, justified the preclinical development of new therapeutics based on these TLR4 modulators. Several lead compounds are now available, efficiently targeting in animal models an array of infective and inflammatory diseases, including neuroinflammatory and neurodegenerative diseases, whose etiology is linked to excessive and deregulated TLR4 activation and signaling.
Active research projects (2014): 1) development of new drug candidates targeting TLR4, 2) study of molecular interactions between MD-2.TLR4 complex and CD14 receptor and natural or synthetic molecules active on TLR4 signal, 3) study of molecular aspects of interaction between LPS transport protein of gram negative bacteria and LPS, development of small molecules interfering with LPS transport in bacteria to have access to new generations of antibiotic.

Abstract

Students

Role of the Late Sodium Current (I_{NaL}) in a cellular model of acute myocardial ischemia

Carlotta Ronchi[1], Riccardo Rizzetto[1], Marcella Roccchetti[1], Antonio Zaza[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

c.ronchi5@campus.unimib.it

Background: The V-gated Na^+ current may be largely inactivated during acute ischemia; but, ranolazine (RAN), a blocker of its late component (I_{NaL}), has been shown to improve recovery during reperfusion. Whether I_{NaL} is present and contributes during acute ischemia is unknown.
Aim: To test the functional contribution of I_{NaL} in a cellular model of acute ischemia.
Methods Isolated rat ventricular myocytes were exposed to an ischemia-mimic solution (ISC), in the absence (CTRL) or in the presence of 10uM RAN (RAN).
Cell shortening (video-edge detection), intracellular Ca^{2+} and Na^+ dynamics (Fluo4AM, ANG-2) were monitored in field stimulated intact myocytes (1Hz). Action potentials (APs) were recorded in patch-clamped myocytes. I_{NaL} was isolated as the current sensitive to 1uM TTX using AP waveforms recorded in pre-ischemia (APctrl) or at 7 min ISC (APisc).
Results: During ISC, transient loss of contraction was followed by partial recovery within 7 minutes, blunted by RAN ($p< 0.05$). ISC induced: 1) progressive increase of diastolic Ca^{2+} (Ca_{diast}) and Ca^{2+} transient amplitude (CaT); 2) Ca^{2+} waves development (80%); 3) transient enhancement of cytosolic Na^+. RAN prevented the increment in CaT, decreased the frequency of Ca^{2+} waves (35%), inhibited cytosolic Na^+ increase but did not affect the Ca_{diast}.
Although APs could be elicited throughout ISC, diastolic potential markedly depolarized and the AP upstroke velocity slowed. Under APctrl, I_{NaL} was present in CTRL and enhanced at 7 min ISC (86%). When APisc was applied during ISC, I_{NaL} was still enhanced (65%). RAN abolished ISC-induced I_{NaL} enhancement.
Conclusion: I_{NaL} contribution to AP increased in spite of AP waveform changes during ischemia. I_{NaL} enhancement participated to partial recovery of contractility, intracellular Ca^{2+} overload, transient increase of cytosolic Na^+ and Ca^{2+} waves development during acute ischemia. Thus, I_{NaL} blockade during acute ischemia may account for RAN protective effect during reperfusion.

Phosphatidylinositol 3-phosphate mimics based on a glucose scaffold

Cipolla L.[1], Gabrielli L.[2], Vetro M.[3], Colombo D.[3], Legnani L.[3],
Costa B.[1], Donvito G.[1], Perego P.[4], **Calloni I.[1]**

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2]*Dipartimento di Scienze Chimiche, Università di Padova, Padova, Italy*
[3]*Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Milan, Italy*
[4] *Dipartimento di Oncologia Sperimentale, Istituto Nazionale dei Tumori, Milan, Italy*

i.calloni@campus.unimib.it

Protein kinase B/AKT plays a central role in cancer. The serine/threonine kinase is over-expressed or constitutively active in many cancers and has been validated as a therapeutic target for cancer treatment. However, targeting the kinase activity has revealed itself to be a challenge due to non-selectivity of the compounds towards other kinases.

Inhibitors that target PI3Ks and its downstream effectors, including PKB are potentially relevant for cancer therapy. PI3K activation generates 3-phosphorylated phosphatidylinositols [PI(3)P] that bind PKB pleckstrin homology (PH) domain promoting PKB activation through its translocation from the cytosol to the plasma membrane, conformational change and final phosphorylation. Several analogs of inositol phosphate has been described that inhibit the binding of the PH domain to PIP(3)P. Given the structural similarity between inositol and glucose, a series of glucose derivatives as mimics of phosphatidylinositols have been synthesized and evaluated in vitro for their activity against PKB.

Acknowledgements. This work has been supported by Fondazione Cariplo, grant n° 2011-0490.

Neuronal cell differentiation on neoglucosylated collagen matrices

Sgambato A.[1], Russo L.[1], Lecchi M.[1], Pastori V.[1], Natalello A.[1], Achilli S.[1], Cipolla L.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

a.sgambato@campus.unimib.it

Glycans are directly involved in almost every biological important process, in fact they have many biochemical structural and functional features, that provide the stimulation or the enhancement of cellular events [1]. Emerging evidence suggests a pivotal role for glycans in regulating nervous system development and function. For instance, glycosylation influences various neuronal processes, such as neurite outgrowth and morphology, and may contribute to the molecular events that underlie learning and memory. The complexity of glycan functions help to orchestrate proper neuronal development during embryogenesis, as well as influence behaviors in the adult organism [2].

In this work we prepared collagen matrices functionalized with glucose moieties at their surface in order to investigate neuroblastoma F11 cell line behavior on grafted glucose. Collagen is usually glycosylated with α-(1→2)-D-glucosyl-β-D-galactosides linked to hydroxylysine residues, where glucose is added as the last residue and most likely elicits a specific biological response. For this reason, we decided to investigate the effect of collagen matrix neoglucosylation on neuronal differentiation. The behavior of cells plated on native and neoglucosylated collagen has been compared with cells seeded on petri dishes. Results show for the first time F11 cells differentiated without the use of conventional differentiating agents and suggest that neoglucosylated collagen is an efficient biomaterial to be employed for neuronal differentiation [3].

Acknowledgements. We gratefully acknowledge Fondazione Cariplo, grant n° 2010-0378, n° 2011-0270 and MIUR, under project PRIN 2010/L9SH3K.

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Lipopolysaccharide transport and peptidoglycan remodelling: two related process in Escherichia Coli

Morè N.[1], Martorana A.M.[1], Sperandeo P.[1], Vollmer W.[2], Polissi A.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2]*Institute for Cell and Molecular Biosciences, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, UK.*

n.more1@campus.unimib.it

The Gram-negative bacteria cell envelope consists of three layers: an inner cytoplasmic membrane (IM) made of phospholipids, an outer asymmetric membrane (OM) containing phospholipids in the inner leaflet and a unique glycolipid lipopolysaccharide (LPS) in the outer leaflet, and a thin layer of peptidoglycan (PG) sandwiched between them. The LPS synthesized in cytoplasm is taken up by the multiprotein complex Lpt (LPS transport), that in Escherichia coli consist of seven essential proteins (LptABCDEFG) which assists its transport until reaching its final location, the OM. PG is anchored to the OM. Recently we performed differential proteome analysis of the total membranes of E. coli upon depletion of the IM LptC protein to study how the cell responds to the block of LPS transport. Among the proteins whose level changes in comparison between the depleted and non-depleted strains, we found proteins involved in biogenesis and remodelling of PG. To investigate the relationship between the block of LPS transport and PG remodelling, we analyzed the structure of PG upon LptC depletion. The analysis of PG sacculi from Lpt depleted cells revealed an increase of the non canonical 3-3 cross-link between adiacent glycan strands, as compared to wild type. The non-canonical crosslinking, 3-3 link, is catalyzed by L,D-transpeptidases. The function of this alternative PG crosslinking is at present unknown. In E. coli YnhG and YcbB have been show to catalyze the 3-3 cross-link. To further investigate the correlation between the 3-3 cross-link and the block of LPS transport we constructed arabinose dependent mutants for some of Lpt system components in combination with deletion of both genes that express the L,D-transpeptidases YnhG and YcbB. We hypothesize that this remodelling leads to rescue the OM stability, as cell mutants impaired in this function undergo to lyses upon Lpt depletion.

Heterobifunctional dendrons for multivalent carbohydrate presentation

Bini D.[1], Vento S.[1], Cipolla L.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

davide.bini@unimib.it

Recognition processes between glycans and their receptors are of paramount relevance in several biological phenomena, both in physiological [1] and in pathological [2] conditions. Beside the variation of carbohydrate residues in glycoconjugates, a key issue in the recognition process is their spatial topographical presentation eliciting high affinity recognition events. In order to better understand these phenomena, dendrimers and dendrons have been developed to provide multivalent glycoconjugates [3, 4]. Here we propose the synthesis of new dendron structures that allow the multivalent conjugation of carbohydrates via carbonyl chemistry.
The synthesis of new dendrons of generation 0, 1 and 2 possessing a double bond at the focal point and a carbonyl group at the termini have been synthesised. The carbonyl group has been exploited for the multivalent conjugation to a sample saccharide via reductive amination and alkoxyamine conjugation.

Acknowledgments. We gratefully acknowledge Fondazione Cariplo, grant n° 2010-0378, n° 2011-0270 and MIUR, under project PRIN 2010/L9SH3K.

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Hydrophobic carminic acid derivatives and their inclusion in organic polymer matrices.

Origgi D.[1], Cipolla L.[1], Gabrielli L.[2], Bonetti S.[3], Simonutti R.[3]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2]*Dipartimento di Scienze Chimiche, Università di Padova, Padova, Italy*
[3] *Dipartimento di Scienza dei Materiali, Università di Milano-Bicocca, Milan, Italy*

d.origgi1@campus.unimib.it

Carminic acid is a natural hydroxyanthraquinoid red pigment [1] possessing a C-glucosyl moiety linked to the antraquinonone scaffold.
Carminic acid is obtained from cochineal (Dactylopius coccus) from the body and eggs of the female insect. Carminic acid extract is very soluble in water and exhibits shade changes with changes in pH. At pH 3 and below, it is orange; it turns from red to violet by increasing pH from 4 to 8. It has a good stability to heat, chemical oxidation, light and oxygen, being more stable than some synthetic food grade colorants but instable at low pH [2]. Cochineal extract and carmine are neither toxic nor known to be carcinogenic.
Natural pigments and colorants are widely used in the world in many industries such as textile dying, food processing or cosmetic manufacturing. From an industrial point of view, the conversion of water-soluble carminic acid to unsoluble forms attracts considerable interest.
In this report, we wish to present different attempts toward the chemical derivatisation of carminic acid in order to modulate its solubility and its inclusion in organic polymeric matrices.

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The ALIAmides: a novel option for the treatment of human osteoarthritis

Donvito G.[1], Costa B.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

g.donvito1@campus.unimib.it

Osteoarthritis (OA) is the most degenerative joint disease that reduces patients' quality of life. Acetaminophen, and NSAIDs are employed for pain relief associated with OA. However, their prolonged use induces serious side effects. So the identification of alternative drugs is exential. For this purpose, in MIA-induced OA rat model, we investigate the anti-inflammatory, and antinociceptive efficacy of palmitoylethanolamide (PEA), the parent molecule of ALIAmides (Autacoid Local Injury Antagonism Amides), a group of endogenous fatty acid derivatives sharing anti-inflammatory and antinociceptive effects through the down-modulation of local mast cell degranulation. In MIA treated rats, PEA was able to completely abolish knee swelling, reduce thermal hyperalgesia, and mechanical allodynia, restore locomotor functionality, and preserve cartilage from damage. Starting from these results, we demonstrated that PEA could represent a viable alternative for the OA treatment. Moreover, we also evaluated the efficacy of Glupamid, that is considered as a PEA analogue. Oral administration of Glupamid 30mg/kg exerted anti-inflammatory, and antinociceptive effects, improved locomotor impairment, compared to MIA rats orally treated with equimolar dose of glucosamine HCl. Another very interesting analogue of PEA is Adelmidrol, belonging for structure and mechanism of action to the ALIAmides. Intra-articular (i.a.) administration of Adelmidrol in MIA-induced OA evoked a dose-dipendent anti-inflammatory and antiallodynic effect, and it was also able to restore motor function. In conclusion, these results suggest that the family of ALIAmides is a variegated class of compounds with different chemical characteristic but their same mechanism of action makes them able to have both anti-inflammatory and antinociceptive properties. For this reason, the ALIAmides represents a valid and effective alternative for the treatment of human osteoarthritis that lacks a resolutive therapy still now.

Functional characterization of Sox4 regulatory elements targeted by the transcription factor Sox2

Barone C.[1], Favaro R.[1], Bertolini J.[1], Delvecchio S.[1], Mercurio S.[1], Martynoga B.[2], Guillemot F.[2], Wei CL.[3], Nicolis S. K.[1].

[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy
[2]MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London, UK
[3]DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Walnut Creek, USA

c.barone6@campus.unimib.it

SOX2 is a transcriptional factor essential for early stages of vertebrate development and CNS development. The Sox2 constitutive Knockout (KO) is embryonic lethal, thus to study the effect of Sox2 absence during neural development we use a conditional KO.
Our interest is to investigate the possible role of Sox2 in regulating disease-linked genes. A list of potential Sox2 target genes was determined crossing data obtained through different approaches. We compared long-range DNA interactions in chromatin of wild-type mouse neural stem/precursor cells (NPCs) and Sox2-deleted NPCs, using the ChIA-PET technique: out of a total of 7000 long-range interactions mapped in wild-type NPCs, 2700 were lost in Sox2-deleted cells. Than we determined differentially expressed genes in wild-type versus Sox2-deleted NPCs, by RNA-seq, and we mapped SOX2 binding sites in chromatin of wild-type NPCs by ChIP-seq.
I investigated the role of Sox2 in regulating one putative Sox2 target: Sox4. This gene is involved in CNS embryonic development, in neural precursors commitment in adult life and is linked to Charge syndrome, a genetic disorder with similarities to the Sox2-mutant phenotype.
Sox4 expression, analysed by In Situ Hybridization and qPCR, appears reduced in Sox2 mutant mice compared to WT both in perinatal and embryonic stages. To investigate if Sox4 could be a direct target of Sox2 I studied the Sox4 promoter and a putative Sox2-dependent Sox4 enhancer, identified by ChIA-Pet. The putative Sox4 enhancer had been shown to have enhancer activity in mice by others and we found that it has enhancer activity in zebrafish.
We observed that both the Sox4 promoter and its putative enhancer can drive transactivation of the luciferase reporter gene in P19 cultured cells, in a Sox2-dipendent manner. In addition, Sox2 shows a synergic cooperation with Ascl1/Mash1 in regulating the Sox4 putative enhancer.
In vivo and in vitro data suggest that Sox2 can have a role in Sox4 regulation.

Role of RalGPS2, a new possible oncogene, in human bladder cancer cells

D'Aloia A.[1], Martegani E.[1], Ceriani M.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

a.daloia1@campus.unimib.it

RalGPS2 is a GEF for the GTPase RalA belonging to RalGPS family that contains a well conserved Ras-GEF domain, a PxxP motif and a PH domain. Previous experiments in our laboratory have demonstrated that there is a partial, but marked co-localization between RalA, PH domain and PH-PxxP region of RalGPS2 at plasma membrane and in thin membrane protrusions. Besides, the overexpression of PH domain and PH-PxxP region induces a marked cytoskeleton re-organization: in particular the PH domain causes formation of thin vesiculating protrusions while the PH-PxxP region causes formation of long inter-cellular structures probably involved in the exchange of signals. The presence of these actin-based protrusions and RalA localization in these structures suggests that these could be tunnelling nanotubes (TNTs). TNTs are a new type of cell-cell communication between remote cells and through TNTs cells can exchange various cellular components and signals. Recent research has shown that the transmembrane LST1 induces TNTs formation by recruiting the small GTPase RalA to the plasma membrane and promoting its interaction with exocyst complex (Schiller et al., 2012).

The aim of this work is to analyze if RalGPS2, its PH-PxxP region and its PH domain play a role in TNTs formation in 5637 cells. Since nanotubes were initially described to contain actin but not tubulin (Rustom et al.,2004), we used this criterion to characterize 5637 protrusions. To analyze the role of exocyst pathway in the formation of these structures we overexpressed the mutant Ral-38R, witch is not able to interact with the exocyst component Sec5, because it is known that RalA and its interaction with components of the exocyst complex are required for TNTs formation (Hase et al,2009). Furthermore we overexpressed LST1-mCherry in 5637 cells and we compared LST1-induced nanotubes with PH-induced protrusions.

Synthesis and charachterization of hexosamine biosynthetic pathway modulators

Ricciardiello F.[1], Palorini R.[1], D'Orazio G.[1], La Ferla B.[1], Chiaradonna F.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

f.ricciardiello@campus.unimib.it

In recent years, tumor cells have been defined "metabolic entities" due to their ability of reprogramming their metabolism to support cancer development also under stress conditions. It has been extensively shown that glucose and glutamine are essential sources for cancer cells. The Hexosamine Biosynthetic Pathway (HBP) converges them with acetyl-CoA and uridine-diphosphate to obtain the UDP-N-acetylglucosamine, the necessary substrate for protein O- and N-glycosylation. More than 50% of cellular molecules are affected by glycosylation and deregulation or truncation of glycosylated chain may lead to abnormal cell behavior causing different diseases. A strong correlation exists between abnormal glycosylation level and increased proliferation, adhesion, motility, stress resistance, as well as angiogenesis and metastasis in cancer cells. For this reason the need to synthetize new HBP modulators, especially inhibitors, is an actual challenge. Here it is reported the synthesis and the first characterization of two novel compounds that should act as specific modulators of phosphate acetylglucosamine mutase (AGM), the enzyme involved in the conversion of N-acetylglucosamine 6-phosphate to N-acetylglucosamine 1-phosphate in HBP. Preliminary results obtained by using both compounds, namely FR_051 and FR_049, in the breast cancer cell line MDA-MB-231 have indicated their ability to induce cell death. Beside, such cell death has been associated to an alteration of membrane protein N-glycosylation levels and to a pronounced cell detachment, suggesting a specific activity of both compounds on HBP pathway. These promising results have to be corroborated by other in vitro and in vivo experiments in order to further characterize the two molecules and possibly hypothesize the design of new compounds. These HBP modulators will be used to deeper investigate the role of the pathway and the possible therapeutic effects of its modulation in cancer cells.

Coral dominated benthic assemblages composition and distribution in Southern Faafu atoll (Maldives): use of family Fungiidae as proxy

Martin C.[1], Hoeksema B.W.[2], Galli P.[1], Benzoni F.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2]*Department of Marine Zoology, Naturalis Biodiversity Center, Leiden, The Netherlands*

c.martin@campus.unimib.it

The composition of coral dominated benthic assemblages in Southern Faafu atoll, Maldives, was investigated and the influence of bathymetry, geomorphology and exposition on their distribution addressed. The coral family Fungiidae was tested as proxy of the distribution of reef assemblages. Assemblages were surveyed at four depths (5, 10, 15, 20 m) and sites chosen between inner and outer reefs with different geomorphologies. Thirteen types of coral dominated benthic assemblages were characterized. The main environmental factor influencing coral dominated benthic assemblages is exposition, with a remarkable distinction between inner and outer reefs. Geomorphology and bathymetry also contribute to the differentiation, showing that faru, coral gardens and upper depths are the richest in corals, while walls have affinity to the outer reefs. Fungiidae proved to be a good proxy for benthic assemblages distribution at shallower depths (5, 10 m).

Characterization of the role of the transcription factor Sox2 in cerebellum development by means of conditional knock out

Bottes S.[1], Mercurio S.[1], Leto K.[2], Concina G.[2], Cau E.[2], Rossi F.[2], Nicolis K. S.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2]*Department of Neuroscience "Rita Levi Montalcini", University of Turin, Turin, Italy*

s.bottes@campus.unimib.it

Sox2 is a transcription factor essential for maintenance of embryonic and neural stem cells. It is expressed throughout the developing neural tube and in neural progenitors in the adult brain. Sox2 knock out (KO) embryos die soon after implantation; therefore, to study Sox2 role in cerebellum development, we ablate Sox2 by conditional KO in mice with a Wnt1-Cre transgene, which remove the floxed gene from embryonic day (E) 10 in midbrain and hindbrain.
The cerebellum derives from rhombomere 1 in the hindbrain and is our coordination center. In humans, cerebellar damages produce disorders in fine movement, equilibrium, posture and motor learning.
The neural tissue at the midbrain-hindbrain boundary, the isthmus organizer (IsO), is required to define midbrain and cerebellar territories.
The Wnt1-Cre mediated sox2 ablation results in behavioral defects in adult mice that include motor coordination defects, balance problems and feet-clasping (collaboration with Ferdinando Rossi Lab, Turin University).
How do these behavioral phenotypes originate?
The aim of this project is to understand if an early developmental defect could be the cause of the behavioral phenotypes observed.
I found that Sox2 ablated embryos present a larger mesencephalon compared to controls. This defect is first detected at E10.5 and still present in adult mice. Furthermore, I observed that the cerebellum is reduced in size and its position is altered compared to controls.
I am currently investigating the expression pattern of cellular and area markers at different developmental stages in order to evaluate possible differences, between controls and mutants, that could explain the morphological phenotype. In particular, I am looking at the expression of Otx2 and Gbx2, markers of midbrain and anterior hindbrain respectively, required to define the position of the IsO and potential Sox2 targets.
We can conclude that Sox2 is involved in cerebellum development, particularly in the early steps.

Characterization of the role of the transcription factor Sox2 in arealization of the cerebral cortex

Gesuita L.[1], Mercurio S.[1], Savidan M.S.[1], Favaro R.[1], Yubo Z.[2], Ottolenghi S.[1], O'Leary D.D.M.[3], Robson P.[4], Wei CL.[2], Nicolis S. K.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2]*Sequencing Technology Group, Joint Genome Institute, Walnut Creek, United States*
[3]*Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, United States*
[4]*Genome Institute of Singapore, Singapore*

l.gesuita1@campus.unimib.it

The mouse neocortex is organized into four functional primary areas; three are associated with sensory functions (visual, somato-sensory and auditory) and one with motor ability. The process of patterning of these areas is called “arealization” and is controlled by two distinct mechanisms. First, it requires the graded expression of four trascription factors (Sp8, CoupTF1, Pax6, Emx2) in telencephalic neural progenitors at early stages of development; the gradients of these transcription factors are mutually interacting and the single knock-down or overexpression of one of them affects the final pattern. Second, the arrival of thalamo-cortical projection from specific thalamic nuclei is essential to better define the borders of each area.
Our interest in arealization began after genome-wide studies performed in our laboratory. These analysis identified Sp8 and Couptf1, important factors in arealization, as potential targets of Sox2, a transcription factor expressed in neural progenitors and in some neurons. We found that Sp8 and CoupTF1 are differentially expressed in Sox2-ablated versus normal neural stem cells (NSCs) through RNAseq. Furthermore, we identified through ChIA-PET long-range interactions (RNApolIII dependent) between these genes and putative regulatory elements that are lost in chromatin of Sox2-ablated NSCs.
To address Sox2 function in the developing neocortex, we generated Sox2 conditional knock-outs. The deletion in the ventricular zone starting at embryonic day (E) 10.5 by an Emx1-Cre knock-in, leads to a 30% reduction of the primary visual area at postnatal day (P)7 and a 50% reduction at P10. On the other hand the deletion in three thalamic nuclei (dorsal lateral geniculate, ventral posterior, medial geniculate) at E 14.5 by a Rorα-Cre knock-in affects both primary visual and sensory areas which appear fuzzy at P7.
Intriguingly, Sox2 seems to have a role in arealization of the neocortex although it has not an evident graded expression.

Drinking water microbiome: towards a method assessing microbial structure viariability

Bruno A.[1], Mezzasalma V.[1], Sandionigi A.[1], Barbuto M.[1], Galimberti A.[1], Cerizzi R.[1], Cocuzza C.[2], Casiraghi M.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2] *Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*

a.bruno2@campus.unimib.it

Drinking water quality is a public health concern worldwide. Growing evidences depict drinking water as a complex matrix, in which a wide diversity of microorganisms interact in a dynamic network. Recent studies reveal that drinking water treatment process can affect the microbiome structure. In particular granular activated carbon filters seem to play a crucial role in shaping bacterial community downstream the treatment processes.
Dealing with environmental samples, the "great plate count anomaly" must be taken into account: only a minimal portion of bacteria can grow on cultured media. Molecular techniques can give a deeper knowledge, going beyond the limit of culture-dependent methods.
We collected samples in a water treatment plant in Milan (Italy), at different steps of the potabilization processes, from the source to the tap. In this study we evaluated and standardized a new pipeline for microorganisms concentration, DNA extraction and amplification, suitable for molecular analysis and optimized for High-Throughput Sequencing approaches.
We further analyzed the presence and the relative abundance of bacteria and eukaryotic microorganisms across the water treatment plant. Moreover the presence of specific antibiotic resistance genes was detected and quantified with real time PCR, at each step of water treatment process. The occurrence of antibiotic resistance genes (ARGs) in water is becoming an issue of great interest as the mobile resistome can easily spread among species. Since molecular techniques are unable to differentiate between viable and nonviable microorganisms, live/dead ratio was estimated using SYTO9/propidium iodide staining coupled with microscopy visualization.
The results agree with those obtained in the few recent studies published till now.
This analysis is integrated in a broader study characterizing microbiome structure variability using High-Throughput Sequencing, in order to better understand this complex ecosystem.

Evaluation of Zygosaccharomyces bailii for lactic acid tolerance

Kuanyshev N. [1], Porro D.[1], Branduardi P.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

n.kuanyshev@campus.unimib.it

Nowadays an industry searches for a cheap and environmentally friendly way to produce fine chemicals. The cell biofactories are an attractive system to fulfill modern requirements for an industrial production of chemicals and among those lactic acid is considered as an oldest and high-volume produced chemical. However, bacterial strains used in lactic acid fermentation suffer from low acid tolerance making downstream purification of final product troublesome and expensive. Exploitation of acid tolerant yeast strains can effectively eliminate the obstacle. Zygosaccharomyces bailii is a well-known food spoilage yeast usually found in can and wine industry. Z. bailii possess a significant low pH tolerance and organic acid tolerance which can be considered as an advantage in lactic acid production. Therefore we would like to elucidate and understand the mechanism underlying lactic acid tolerance in Z. bailii not thoroughly investigated up to now. Data resulting from several fermentations using both flasks and bioreactor with different pH, oxygen supply and organic acid concentrations will be presented and discussed, together with evaluations regarding viability profile measured at single cell level.

Optimization of lipid accumulation in oleaginous yeasts using pure and crude glycerol

Signori L.[1], Porro D.[1], Branduardi P.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

l.signori@campus.unimib.it

Biodiesel is usually produced from food-grade plant oils using transesterification process; however this kind of production is not economically feasible since they are more expensive than diesel fuel. Microbial lipids can represent a valuable alternative feedstock for biodiesel production and a potential solution for a biobased economy. It has been well established that fatty substances are produced by various microorganisms, notably by certain yeasts and fungi. Oil yeasts have been described to be able to accumulate lipid up to 20% of their cellular dry weight and, among these, few have been reported to accumulate oil up to 80%. Oleaginous yeasts could accumulate intracellular lipids by cultivation on various agro-industrial wastes as crude-glycerol, a 10% (w/w) byproduct produced in the transesterification process of oils converted to biodiesel. It is well reported in literature that different oleaginous yeast strains present different metabolic responses depending on the origin of the crude glycerol employed, which may result in inhibitory effect on the yeast cells growth.

The present work studied crude-glycerol as carbon sources for lipid production using two different oleaginous yeasts: Rhodosporidium toruloides (DSM 4444), and Cryptococcus curvatus (DSM 70022).

The main objective was to develop a successful fermentative strategy for achieving a high lipid productivity avoiding detrimental and inhibitory effects of crude-glycerol. Moreover, a set of analysis at single cell level were performed to evaluate lipid accumulation (Nile-Red staining) over time of the batch cultures. Reported results show a correlation between lipid accumulation and an increase in fluorescence over time and also fluorescence microscopy confirmed this trend. Further analysis, including Cell Dry Weight and HPLC measurements, will be presented and discussed in the view of a deeper understanding of the growth and lipid accumulation capabilities of these two yeasts.

mRME (mRNA metabolic engineering): a novel approach to obtain industrial phenotypes

Marano F.[1], Martani F.[1], Bertacchi S.[1], Porro D.[1], Branduardi P.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

f.marano@campus.unimib.it

Biotechnological processes are of increasing significance for industrial production of fine and bulk chemicals, including biofuels. Under operative conditions microorganisms meet multiple stresses such as non optimal pH, temperature, oxygenation and osmotic stress. Moreover, they have to face inhibitory compounds released during the pre-treatment of lignocellulosic biomasses, which constitute the preferential substrate for second generation production processes. All together these factors impair cellular metabolism and growth and, as a consequence, reduce the productivity of the process.

The highly desirable evolution of robust cell factories is rarely ascribable to a single molecular element, since it requires a complex cellular reprogramming, implying the simultaneous modification of many regulatory and operative elements. In addition to transcription, cells can modulate their complex phenotype by controlling mRNA metabolism and trafficking, translation and finally post-translational modifications. During stressful conditions the translational machinery slows down and the mRNAs are aggregated in cytoplasmatic ribonucleic foci, known as stress granules, where transcripts are stored until the cell has been adapted to the stress. Poly(A) tail length represents an important threshold for mRNAs rate of degradation or translation. Pab1 is the yeast major poly(A) binding protein, playing an important role in mRNA metabolism modulation, and it is also a known component of stress granules.

Here we present our approach for manipulating post-transcriptional events in the yeast cell factory Saccharomyces cerevisiae by modulating and mutagenizing PAB1 as a key regulatory element.

From oral probiotics to vaginal colonization

Enrico Manfrini[1], Valerio Mezzasalma[1], Antonia Bruno[1], Fabrizio De Mattia[1], Ilaria Bruni[1], Emanuele Ferri[1], Emanuele Panunzi[1], Massimo Labra[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

e.manfrini@campus.unimib.it

Each human body plays host to a microbial population which is both numerically vast (at around 10^14 microbial cells) and phenomenally diverse (over 1000 species). The human microbiome resides on the skin, in the oral cavity, in the conjunctiva, in the gastrointestinal tracts and in the female genital tract.
The vaginal microflora is composed of many bacterial species and plays a major role in maintaining the balance of this complex environment.
This study was conducted in order to assess the degree and persistence of the colonization of vaginal epithelium by strains from an orally administered mixture, compared to placebo in healthy women. This study includes two probiotics capsules (Mix1 and Mix2) cointaining strains of Lactobacillus spp. and Bifidobacterium spp. isolated from faeces of healthy humans. The study consisted in daily administration of one mixture capsule for 14 days with four vaginal swab collections at T0, T7, T14 and, seven days after last capsule assumption, T21. The volunteers were 60 healthy women, 20 for each group: Mix1, Mix2 and placebo.
Genomic DNA was extracted from the vaginal swab and analyzed by relative quantitative Real Time PCR, with species-specific designed oligonucleotides.
We confirmed that the probiotic strains were able to reach and colonize vaginal site. Moreover probiotic colonization persists also after seven days without mixture capsule ingestion. No adverse events were noted during the course of the study. Oral administration of the combination of the probiotic strains derived from gut microbiota shows abilities to colonize vagina for some weeks.
Since vaginal microbiota has a very important role against bacterial vaginosis, yeast infections and other pathogens colonization, it will be very interesting to analyze the effect of orally administered probiotic in preventing and reducing risk of pathogen infections.

Protein nanocages for self-triggered nuclear delivery of DNA-targeted chemotherapeutics in Cancer Cells

Bellini M.[1], Mazzucchelli S.[2], Galbiati E.[1], Sommaruga S.[2], Fiandra L.[2], Truffi M.[2], Rizzuto M. A.[1], Colombo M.[1], Tortora P.[1], Corsi F.[2], Prosperi D.[1]

[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[2]*Dipartimento di Scienze Biomediche e Cliniche "Luigi Sacco", Università di Milano, Ospedale L. Sacco, Milano, Italy*

m.bellini16@campus.unimib.it

Ferritin is an iron storage protein composed of a regular assembly of 24 subunits to form a spherical cage architecture, which plays a key role in the metabolism of iron, protecting the cell from oxidative stress. Apoferritin nanoshell could be a good candidate nanocarrier for drug delivery in tumors, thanks to the opportunity to encapsulate different molecules within the cavity and for the ability to recognize cancer cell overexpressing the transferrin receptor 1 with high specificity and high sensitivity. A genetically engineered apoferritin variant consisting of heavy-chain subunits (HFn) was produced to achieve a cumulative delivery of doxorubicin, which exerts its cytotoxic action by targeting the DNA. The rationale of our approach is based on exploiting the natural arsenal of defense of cancer cells to stimulate them to recruit large amounts of HFn nanoparticles loaded with doxorubicin inside their nucleus in response to a DNA damage. After demonstrating the selectivity of HFn for representative cancer cells compared to healthy fibroblasts, doxorubicin-loaded HFn was used to treat the cancer cells. The results from confocal microscopy and DNA damage assays proved that HFn(DOX) increased the nuclear delivery of the drug, thus enhancing doxorubicin efficacy. HFn(DOX) acts as a "Trojan Horse": HFn was internalized in cancer cells faster and more efficiently compared to the free drug, then promptly translocated into the nucleus following the DNA damage caused by the partial release in the cytoplasm of encapsulated doxorubicin. This self-triggered translocation mechanism allowed the drug to be directly released in the nuclear compartment, where it exerted its toxic action. This approach was reliable and straightforward providing an antiproliferative effect with high reproducibility. The self-assembling nature of HFn nanocage makes it a versatile and tunable nanovector for a broad range of molecules suitable both for detection and treatment of cancer cells.

Characterization of GLT1 and GDH1 modulation on Saccharomyces cerevisiae metabolism and physiology

Brambilla M., Adamo GM., Porro D., Branduardi P.

Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

m.brambilla60@campus.unimib.it

In a biorefinery, different types of biomass are converted into sugars that are used as carbon source by microorganisms, called cell factories, to produce chemicals and biofuels. Saccharomyces cerevisiae is one of the most characterized cell factory, employed in different biotechnological applications. Considerable efforts are oriented to the study and the engineering of carbon assimilation by S. cerevisiae, in order to achieve a high production, productivity and yield of the bioprocess. However, the study and the eventual engineering of nitrogen assimilation are also of great importance to design cell factories with an improved capability to exploit proteins-rich biomasses in fermentative processes.
In S. cerevisiae, nitrogen compounds, once inside the cells, are catabolized through the Central Nitrogen Metabolism (CNM), in which the enzymes glutamate dehydrogenase 1 (Gdh1p), glutamate dehydrogenase 2 (Gdh2p), glutamine synthetase (Gln1p) and glutamate synthase (Glt1p) take part. The effects of GLT1 deletion and over-expression, alone or in combination with GDH1 deletion, on nitrogen assimilation and cell physiology have been characterized in the presence of different nitrogen sources.
Results are shown and discussed, together with future perspectives.

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http://btbsday.btbs.unimib.it
btbsday@unimib.it