



2012 SUMMER STUDENT PROJECT DESCRIPTIONS

PROJECT 1

Supervisor: Prof. Silvia Barabino
Department: Biotechnology and Biosciences
Laboratory: Milan

Line 1 Mechanisms of post-transcriptional regulation of mammalian gene expression and their role in human disease - Cell stress and RNA splicing

Coupling of pre-mRNA splicing to extracellular signals is crucial for altering splicing patterns according to the physiological state of cells. We have recently established a cellular model that will allow us to elucidate the molecular changes in the alternative splicing machinery induced by the oxidative stress response. Oxidative stress arising from mitochondrial dysfunction has been proposed as concurring to the pathogenesis of many neurodegenerative diseases, including Parkinson Disease and Amyotrophic Lateral Sclerosis (ALS). Defects in the splicing of individual mRNAs have also been observed in the affected tissues of ALS patients. Based on these observations we are investigating in our cellular model whether oxidative stress can induce aberrant alternative mRNA processing thus contributing to the development and the progression of ALS.

Line 2 Epigenetic and RNA processing – Role of Chromatin structure in the regulation of alternative splicing

Alternative splicing plays critical roles in differentiation, development, and disease and is a major source for protein diversity in higher eukaryotes. Recent studies point to a key function of chromatin structure and histone modifications in alternative splicing regulation. This project focuses on the study of the role of the chromatin remodelling factor Brahma in the regulation of the choice of alternative terminal exons. It aims at the elucidation of the interaction between chromatin, splicing factors and components of the polyadenylation complex. The study exploits molecular and biochemical techniques such as chromatin immunoprecipitation, quantitative PCR and protein purification techniques.

PROJECT 2

Supervisor: Dr. Silvia Brunelli
Department: Experimental Medicine
Laboratory: Monza

Role of Necdin in skeletal muscle regeneration and in the differentiation of mesoangioblast stem cells

Necdin is a member of the MAGE family that is expressed in developing and perinatal skeletal muscle, in particular in activated satellite cells. We have shown that in muscle necdin increases expression of myogenin, by cooperating with MyoD in the transcription of Myogenin promoter and accelerates differentiation, and counteract satellite cells apoptosis in the damaged muscle. We have also demonstrated that necdin is selectively expressed in the atrophic muscles of cachectic mice (tumor induced cachexia) and formally proved that its expression is causally linked to a protective response of the tissue against tumor-induced wasting, inhibition of myogenic differentiation and fiber regeneration. To get insights into the molecular function of necdin in myoblast differentiation and survival we will further investigate necdin interaction with the TNFalpha pathway at the cellular and molecular level in activated satellite cells on single fiber. We are examining TNFalpha role in cell death and in inhibition of myogenic differentiation and study the crosstalk of specific downstream mediators of its action (p53, caspases) with the necdin-dependent signalling. In addition, the role of the necdin and the p53/sirtuin pathway in the control of cell-quiescence vs self-renewal will be investigated.

PROJECT 3

Supervisor: Prof. Guido Cavalletti

Department: Neuroscience and Biomedical Technologies

Laboratory: Monza

Chemotherapy-induced peripheral neuropathy: new in vivo models in mice

The use of antineoplastic drugs has markedly improved the prognosis of cancer patients. However, a severe and clinically-relevant problem in the administration of several of these compounds is represented by their side effect. A significant proportion of effective agents can be neurotoxic. In these cases the dorsal root ganglia and the peripheral nerves are the most common sites of damage, since the central nervous system is protected by an effective blood-brain barrier. Despite the well-established

clinical and experimental observation that several antineoplastic drugs induce peripheral neurotoxicity, the fine mechanisms of this side effect is unclear, particularly in view of the absence of cell replication in normal adult neurons which should protect them from anti-mitotic drugs.

We are now establishing a completely new set of mice models which will allow to investigate in the same experiment both the anticancer activity and the peripheral neurotoxicity of currently available as well of new anticancer drugs.

PROJECT 4

Supervisor: Dr. Anna Maria Colangelo
Laboratory: Milan

Molecular mechanisms of neurodegeneration and neuroprotection by Nerve Growth Factor (NGF) and antioxidant molecules

Based on our modular (Systems Biology) model of neuronal apoptosis, mitochondria represent the central core of neuronal dysfunction (Alberghina L & Colangelo AM, 2006 and 2010; Bianco et al., 2011). Moreover, our studies in models of peripheral nerve injury emphasize the role of reactive gliosis plays in modulating neuroglial networks and plasticity during neurodegenerative processes (Colangelo et al., 2008, 2011; Cirillo et al., 2010, 2011, 2011b).

We are currently using *in vitro* neuronal systems (primary cortical neurons and neuronal PC12) and models of reactive gliosis, and animal models of peripheral nerve injury to: *i*) analyze molecular and epigenetic mechanisms underlying neuronal dysfunction (survival, mitochondrial function, ROS production, autophagic flux and metabolism, etc); *ii*) mechanisms contributed by reactive astrocytes; *iii*) evaluate neuroprotection by Nerve Growth Factor (NGF), NGF-like peptides and synergic activity of antioxidant molecules.

PROJECT 5

Supervisor: Prof. Condorelli

Line 1 – Heart failure

Main objectives:

- 1) Development of innovative diagnostic systems for the identification of the genes underlying primary cardiomyopathies.
- 2) Identification of target molecules of potential new isotropic drugs; creation of murine models which mimic the human disease.
- 3) Study of biological factors responsible for cardiac failure in humans through the identification of new biomarkers of disease.
- 4) Role of MicroRNAs in cardiovascular disease

Line 2 – Stem cells in cardiac and vascular pathologies

Main objectives:

- 1) Use of stem cells for the generation of cardiomyocytes.
- 2) Analysis of numerosity and impaired migratory/angiogenic capacity of endothelial progenitor cells in coronary disease and myocardial infarction; study of the molecular mechanisms involved and definition of prognostic markers.

Line 3 – Genetics of complex cardiovascular diseases

Main objectives:

1) Identification of genetic variants associated with coronary atherosclerosis and essential hypertension and their interaction with risk factors.

The activity is mainly focused on the identification of the genic variants involved in phenotypes or complex diseases, such as the cardiovascular ones. This is now possible thanks to the advancements in the study of human genome, which enable a fast and deep analysis of an enormous number of polymorphisms. This may be followed by a correlation between genotype and risk factors or clinical variables. For atherosclerosis, some innovative approaches have then been studied for the improvement of endothelial function, which plays a dominant role in its etiopathogenesis. The approach employed is always the GWAS one.

PROJECT 6

Supervisor: Prof. Luca De Gioia

Department: Biotechnology and Biosciences

Laboratory: Milan

Line 1 - Computational studies of protein-ligand interactions

Computer-Aided Drug Design (CADD) has an increasingly important role in simulating drug-receptor interactions, whose comprehension requires a deep understanding of biophysical and biochemical properties of both the ligand and the protein target at an atomic level. The study of the interactions (docking) between a ligand (possibly a drug molecule) and its protein target will be performed at different levels of accuracy: rigid docking (protein structure is held fixed and ligand can freely rototranslate around it) will be employed for screening of large virtual libraries of organic compounds (generated in silico by one of the subroutines of DELOS platform), in order to preliminarily sort out bad (non-interacting) molecules, whereas more sophisticated approaches (MM, MD, Simulated Annealing) will be used to determine and refine more realistic ligand-receptor complex structures.

Line 2 - Computational Bioinorganic Chemistry

The research is oriented toward the dissection of catalytic mechanism of proteins containing metallic cofactors, as well as of their synthetic models. Particular interest is devoted to the mechanism of activation of small molecules such as hydrogen (H₂) and hydrogen peroxide (H₂O₂). The former activity is performed by hydrogenases (Fe-Fe and Ni-Fe, according to the different ions being in the cofactor) whereas the latter is carried out by vanadium haloperoxidase (VHPO).



Line - 3 Molecular dynamics of proteins

Molecular Dynamics (MD) Simulations will be used with the aim of investigating structure-function relationship in enzymes and proteins. In fact, long and multiple simulations of biomolecular systems can allow to obtain insights into biomolecular processes at the atomic level, which are often hardly accessible to experimental methods.

Particular attention will be addressed to enzymes isolated from cold-adapted organisms. These enzymes are generally characterized by high flexibility, low thermal stability and high specific activity at low temperatures. The project is also aimed at developing computational tools to deal with the huge amount of data which can be obtained from MD simulations.

PROJECT 7

Supervisor: Dr. Maria Foti

Department: Department: Biotechnology and Biosciences

Laboratory: Milan

Dendritic Cell Biology and Molecular Medicine

Development of innate and adaptive immune response during the course of a microbial infection is dependent upon early interactions between incoming microorganisms with immature dendritic cells (iDCs) which are the first immune cells interacting with the microbial agents. The recent improvements of sequencing technologies, and in particular the publication of the initial version of the human and mouse genome sequences, have opened the field of large-scale functional approaches of biological systems. We employ high-throughput technologies to investigate fundamental aspects of the immune system and their roles in health and disease. In order to identify key cellular genes involved in these processes, we use a transcriptomic approach in which modifications of cellular transcriptome are analysed at several times post-infection.

PROJECT 8

Supervisor: Prof. Carlo Gambacorti

Department: Clinical Medicine and Prevention

Laboratory: Monza

Experimental validation of oncogenic fusion genes and other early events in cancer

Most cancers develop from the sequential accumulation of several genetic abnormalities, resulting in the activation/inactivation of different pathways. Therefore, an important therapeutic effect may need the combined inhibition of more than a single oncogene. Colorectal cancer (CRC) is characterized by well-defined

genetic abnormalities. Over 85% of sporadic CRC carry mutations that hyper-activate the Wnt pathway, leading to abnormal β -catenin-dependent gene expression. However, β -catenin targeting fails to kill the cells. This may be due to the fact that CRC carry a variety of additional mutations which appear to be relevant for survival. For instance, RAS/RAF pathway activating mutations are present in >70% of CRC. Given the importance of multiple genetic defects in CRC onset and progression, combined targeting of more than one oncogene may provide superior therapeutic effects compared to hitting a single target.

The therapeutic relevance of various proteins involved in CRC transformation will be assessed in this project. We will study (*in vitro* and *in vivo*) the consequences of RNAi-mediated silencing of β -catenin, KRAS, BRAF, BCL9L, TCF7, TCF7L2 and ITF2, alone or in combinations, in CRC cells. We aim to determine whether accurate knowledge of the relevant genetic alterations present in a transformed clone can be translated into an effective therapeutic approach. These data should provide important insights into future therapeutic strategies to combat colon cancer.

PROJECT 9

Supervisor: Prof. Francesca Granucci
Department: Biotechnology and Biosciences
Laboratory: Milan

Line 1 - Dendritic cells and Natural Killer cells

Natural Killer (NK) cells exert a direct anti-tumor and anti-microbial effect and can influence the development of adaptive T cell responses. Activation of NK cells is regulated by accessory cells such as dendritic cells (DC). Following activation, NK cells accumulate at the lymph nodes draining the site of infection, the key place in which DC and NK cell interactions occur. Taking advantage of the two-photon intravital microscopy technology the capacity of activated NK cells to reach the draining lymph nodes is investigated together with the DC-derived signals necessary for NK cell priming in inflammatory conditions induced by lipopolysaccharides.

Line 2 - Dendritic Cells and regulation of Immune Tolerance

The immune system of vertebrate animals has the capacity to respond to perturbations (invading pathogens, stress signals) limiting self-tissue damage. Tolerance to tissue antigens is achieved through a combination of thymic and peripheral events that eliminate or inactivate potentially dangerous T cells. Several mechanisms have been proposed to explain the induction of tolerance in peripheral autoreactive T cells. Taking advantage of different transgenic and knock out mouse models the mechanisms through which dendritic cells induce T cell tolerance in peripheral lymphoid organs are investigated.



PROJECT 10

Supervisors: Prof. Marina Lotti, Dr Stefania Brocca,
Department: Biotechnology and Biosciences
Laboratory: Milan

Line 1 - Conformation and function determinants of proteins

To understand how function and conformation of proteins are related, we use a combined approach employing mutagenesis strategies, biochemical assays and biophysical techniques enclosing Fourier Transform infrared spectroscopy and nanoelectrospray-ionization mass spectrometry (nano ESI-MS) performed in partner laboratories (S.M. Doglia and R. Grandori from this Department). The effect of protein sequence as well as of posttranslational modifications (i.e. glycosylation, phosphorylation) is also investigated. Among proteins employed as model are enzymes, in particular microbial lipases, and disordered proteins from yeast cell cycle. Moreover, novel biocatalysts are isolated from non commercial sources or produced by protein engineering.

Line 2 - Molecular bases of yeasts adaptation to heavy metal

Cells of *Saccharomyces cerevisiae* and from other related yeast species, exposed heavy metal represent our model to study the physiology and the molecular events occurring during the exposition to metals. This choice is supported by the high degree of conservation of cellular and molecular processes between higher eukaryotes and the yeast *Saccharomyces cerevisiae*. This research is aimed to study the complex process of adaptation to heavy metal and to characterize effects thereof, through a multidisciplinary approach. Therefore, classical techniques of growth and viability assessing are applied to yeast cells besides biochemical and biophysical techniques.

PROJECT 11

Supervisor: Prof. Marialuisa Lavitrano
Department: Surgical Sciences
Laboratory: Monza

Molecular Medicine and Animal Biotechnologies for Successful Organ Transplantation: Study of Hyperacute and Chronic Rejection in Xenotransplantation.

Transplantation is the therapeutic option of choice in case of end stage organ failure, in particular for kidney, liver, pancreas, heart and lung. However, a major limitation is the shortage of organs resulting in extensive waiting lists. There are also two other major limitations of successful organ transplantation: the ischemia/reperfusion injury of the organ and the chronic rejection. Objectives of the research project are to 1)

produce alternative source for organ transplantation with 2) long-term function and survival of xenotransplanted organs. Pigs are considered as optimal source of organs for human transplantation, but this is limited by hyperacute and acute vascular rejection processes. Selected human genes preventing inflammation, thrombosis and apoptosis in xenotransplanted organs will be overexpressed in porcine cells and organs. This project would allow generation of a multi-gene transgenic pig as source of organs or cells for transplantation in humans

PROJECT 12

Supervisor: Prof. Raffaella Meneveri
Department: Experimental Medicine
Laboratory: Monza

Functional genomic approaches to dissect molecular basis of Facioscapulohumeral Dystrophy (FSHD)

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of autosomal dominant muscular dystrophy and it results from deletion of a critical number of D4Z4 repeats on the subtelomeric region of chromosome 4q. A leading hypothesis of FSHD pathogenesis has been that contractions within the repeat array affect local chromatin structure or function, leading to abnormal expression of genes adjacent to the deletion (ANT1, FRG1 and FRG2). More recently, progressive muscle degeneration was observed in transgenic mice that over-express FRG1. However, in FSHD patients the increased expression of FRG1 has not been a uniform finding, and other mechanisms of transcriptional de-regulation, such as improper localization of the 4q telomere in the nucleus, have been proposed. In this regard, the observed deregulation

of 4q35 gene expression in FSHD could also be explained by loss of higher order of chromatin organization in the interphase nucleus. Recently we have found that a dynamic chromatin remodeling of the FSHD locus occurs during human myogenesis and this phenomenon involves the chromatin recruitment of Polycomb repressor complex (PcG) on D4Z4 repeats. In order to dissect the molecular mechanism wide gene expression analysis using Affymetrix microarray has been carried out on human muscle stem cells derived from FSHD patients and healthy controls as cellular models of the FSHD disease. The results of this analysis is in progress. In addition ChIP on chip assays with PcG proteins comparing muscle stem cells derived from FSHD and controls will be performed.



PROJECT 13

Supervisor: Prof. Silvia Nicolis
Department: Biotechnology and Biosciences
Laboratory: Milan

Roles of the Sox2 transcription factor in neural stem cells

Neural stem cells maintain themselves through self-renewal, and give rise by differentiation to neurons and glia. By these properties, they are fundamental for brain development, and raise much hope for regenerative medicine. Altered function of neural stem cells can lead to disease, as seen with neural cancer stem cells. Sox2 is a transcription factor important for embryonic, neural, and other stem cells. To investigate its function, we generated mice carrying a conditional mutation in Sox2 (Sox2 flox), that makes the gene conditionally deletable by Cre recombinases. The student will work with one of these approaches: i) study of mutant brain development, neurogenesis, and gene expression, by in situ hybridization and immunohistochemistry; ii) molecular investigation of Sox2 target genes, by transfection, chromatin immunoprecipitation, and “3C”; iii) study of mutant neural stem cell cultures and rescuing experiments with lentiviral vectors encoding identified target genes.

PROJECT 14

Supervisor: Prof. Marco Parenti
Department: Experimental Medicine
Laboratory: Monza

Identification of brain structural and functional abnormalities in genetic mouse models of Autism Spectrum Disorders (ASDs).

The project will investigate the processes leading to neuronal maturation and formation of fully functional synapses in primary cultures of hippocampal neurons obtained from the brains of wild type and four different knockout mouse strains carrying ASD-like behavioural deficits. Fluorescence and confocal microscopy, timelapse videomicroscopy, and morphometric analysis on fixed/living neurons labelled with selected axonal and synaptic markers will be employed to monitor neurite outgrowth and motility, maturation of pre- and post-synaptic compartments, development of excitatory glutamatergic and inhibitory GABAergic synapses. An artificial synapse formation assay will also be performed, where hippocampal neurons will be co-cultured with non-neuronal cells expressing a cell-adhesion molecule (neuroligin, neurexin) The assay will test whether the cell-adhesion molecule induces the neurons to form stable junctions with synapse-like properties with the nonneuronal cells.



PROJECT 15

Supervisor: Prof. Alessandra Polissi
Department: Biotechnology and Bioscience
Laboratory: Milan

Lipopolysaccharide biogenesis in Gram-negative bacteria

The surface of bacterial pathogens is the first site of host interaction and it is also a major target for antibacterial activity of the host. Among the microbial components, lipopolysaccharide (LPS) in the outer membrane (OM) of Gram-negative bacteria is a key structure that is sensed by the host and represents a potent stimulant of the immune response. LPS biogenesis is therefore a key pathway essential for cell life and pathogenicity. The LPS biogenetic pathway will be dissected by cellular, mutational and functional studies. These studies will focus both, on the model organism *E. coli* and also *Pseudomonas aeruginosa*, an opportunistic pathogen that causes a wide variety of infections in compromised hosts. We will study how the protein machinery that regulates LPS transport to the OM is assembled, by analyzing protein-protein interactions in wild type and mutant cells in which LPS biogenesis is inhibited. We will also determine, by crystallographic studies, the structures of wild type and mutant KdsD, LptC and LptA, all key proteins involved in the LPS biosynthetic (KdsD) or transport (LptA and LptC) pathways.

PROJECT 15

Supervisor: Dr. Davide Prosperi
Department: Biotechnology and Bioscience
Laboratory: Milan

Biofunctionalized magnetic nanoparticles as nanostructured material for the isolation and recycling of catalytic enzymes

We developed hybrid magnetic and/or fluorescent nanoparticles as diagnostic agents for biosensing and preclinical investigation, and for biological application, including protein purification and enzyme recycling. Our research is aimed at nanoparticle synthesis, functionalization with organic and inorganic molecules, characterization (TEM, FTIR, DLS, HRMAS NMR, magnetic relaxivity), *in vivo* and *in vitro* studies on cells and small animals. In particular, the project proposed here is focused on the preparation of dual-mode nanoparticle with a nickel(II) nitriloacetic acid (NTA)-modified Fe₃O₄ core (Fe₃O₄-NTA-Ni₂₊), which enables a one-step protein purification through binding to His-tagged proteins. The specific bond is indeed obtained by peptide modification with a His-tagged domain of the recombinant protein. This dualmode nanoparticle probe should prove to be widely useful in a variety of protein bioassay and interaction/recognition experiments.

PROJECT 17

Supervisor: Prof. Antonella Ronchi
Department: Biotechnology and Bioscience
Laboratory: Milan

Functional profiling of erythroid differentiation/maturation in mouse hematopoiesis

Erythropoiesis is the process of progressive cell differentiation and maturation leading to the production of terminally differentiated erythrocytes that synthesize the globin chains required at different stages of development. Failure of the fine tuning regulation of this process is often cause of disease, such as leukemias and hemoglobinopathies.

To identify new genes controlling erythroid differentiation we carried out a gene expression profiling (by DNA microarrays) on FACS sorted murine fetal liver cells populations at different stages of erythroid differentiation. Among differentially expressed genes, we selected few candidates for further functional assay by overexpression and/or downregutaion (lentiviral vector delivery) in primary mouse and human hematopoietic cells undergoing in vitro erythroid differentiation and in immortalized cell lines. The phenotype of the transduced cells is analysed by the use of molecular (ChIP, RT-PCR) and cellular (in vitro differentiation, colony assays, immunostaining, FACS analysis) assays.

PROJECT 18

Supervisor: Prof. Andrea Biondi
Department: Clinical Medicine and Prevention
Laboratory: Monza

New immunotherapy approaches for leukemias

Chimeric receptors (CAR) molecules have recently emerged as a powerful and attractive tool to redirect T-cell specificity and functional activity against tumors, rendering CAR-manipulated T cells potent players in cancer adoptive immunotherapy. CAR are artificial molecules constituted by an extracellular-antigenbinding domain -consisting of the variable chains of a monoclonal antibody (scFV)- and an intracellular-signalling region -CD3 zeta- that is immediately triggered after antigen recognition, leading to T cell activation, with consequent killing of target cells and cytokine release.

Several CARs have been described so far, directed against various tumoral antigens. Our group is focused on the development and optimization of CAR-mediated approaches for the targeting of different haematological malignancies, including acute myeloid leukaemia (AML), chronic lymphocitic leukaemia (B-CLL), and acute lymphoblastic leukaemia (B-ALL), through T cells expressing CAR specific for the



CD33, CD23 and CD19 antigens, respectively. In fact, for all this kind of leukaemias, a consistent number of patients are still refractory or relapse after standard treatments, therefore supporting the development of innovative anti-tumoral approaches. The main goal of the project will be to analyse the efficiency of the CAR-based immunotherapy approach *in vitro* and *in vivo*, in murine xenogenic models of AML, where we can establish the anti-CD33 redirected T cells activity not only against AML leukemic cells but also against AML-initiating cells and on normal myelopoiesis, and in murine xenogenic models of B-CLL. The final answer concerning the efficacy of this approach will be given by a phase I clinical study with anti-CD19 CAR transduced T cells in transplanted relapsed ALL, from which we expect to obtain relevant information concerning the clinical safety and efficacy of this approach.

PROJECT 19

Supervisor: Prof. Massimo Masserini
Department: Experimental Medicine
Laboratory: Monza

Nanoparticles for therapy and diagnosis of alzheimer disease (nad)

A hallmark of the disease in the AD brain is extracellular aggregates (plaques) of the cytotoxic peptide β -amyloid ($A\beta$). We intend to use nanoparticles (NPs) such as Solid Lipid NPs and Liposomes for therapy and diagnosis, singly or combined (theranostics), focusing on brain $A\beta$ as the target. Brain and blood $A\beta$ are in equilibrium across the blood-brain barrier (BBB), so we consider also blood $A\beta$ as a target.

Different NPs (liposomes, solid lipid NPs, polymeric NPs) will be multifunctionalized with:

- Molecules interacting with $A\beta$,
- Molecules stimulating BBB crossing,
- PET or MRI contrast agents.

Artificial and cellular models will be used to improve and fine-tune NP binding to $A\beta$ and BBB crossing. In addition biocompatibility and physical stability of NPs will be investigated.

PROJECT 20

Supervisor: Dott. Paola Coccetti
Department: Biotechnology and Bioscience
Laboratory: Milan

The role played by protein kinase CK2 in controlling mitosis in *Saccharomyces cerevisiae*

CK2 is a highly conserved protein kinase ubiquitously distributed among eukaryotic cells. In budding yeast, CK2 activity is correlated with growth rate (1) and its inactivation leads to arrest of cell cycle progression with both pre- and post-synthetic DNA content. Several targets of CK2 have been found to serve essential functions. Specifically, Cdc37, a Hsp90 co-chaperone, the cyclin dependent kinase Cdc28, the inhibitor Sic1 and the E2 ubiquitin-conjugating enzyme Cdc34 are relevant CK2 substrates (2-5). Although the G1 arrest of CK2 defective mutants has been shown to be largely dependent upon Sic1 upregulation and increased stability (5,6), no data are so far available to explain why CK2 inactivation blocks the cells during mitosis. The main scientific object of the current proposal is to discover the role played by CK2 in mitosis considering that a lot of CK2 consensus sites, determined by bioinformatic analysis, can be detected in several proteins involved in regulation of mitosis. Identification of new putative targets of CK2 could supply relevant information about novel undiscovered biological functions of CK2. We will investigate if the proteins involved in mitosis are substrates of CK2 mapping the sites of phosphorylation and by site-directed mutagenesis we will verify the physiological relevance of CK2 phosphorylations on their functions. In vivo studies will be run on appropriate mutant strains and in synchronous populations.

PROJECT 21

Supervisor: Dott. Paola Fusi
Department: Biotechnology and Bioscience
Laboratory: Milan

Characterization of NEU3 interaction with EGFR

In recent years many data have suggested a role for human sialidase NEU3 in tumorigenesis, particularly in colorectal cancer: NEU3 localization within the plasma membrane has been shown to depend on EGF stimulation, NEU3 overexpression has been shown to stimulate EGFR phosphorylation and NEU3 has been demonstrated to directly activate EGFR. However the molecular details of the role of this sialidase in colorectal carcinogenesis are still largely unknown.

In this project we propose to investigate the interaction between human NEU3 and EGFR at molecular level. Cross-linking experiments will be performed in transiently transfected HeLa cells overexpressing human sialidase NEU3, in order to covalently link NEU3 and EGFR; the complex will subsequently be purified and analyzed by mass spectrometry. Data obtained through mass spectrometry will be matched with NEU3 three-dimensional structural model, previously obtained in our laboratory using human NEU2 crystallographic structure as a template. Site-directed mutagenesis performed on NEU3 will subsequently validate mass spectrometry results.



PROJECT 22

Supervisor: Dott. Roberta Frascini
Department: Biotechnology and Bioscience
Laboratory: Milan

Mechanisms controlling mitosis and cytokinesis in response to morphological defects and replicative stress in *Saccharomyces cerevisiae*

The overall goal of our research is to improve our understanding of the molecular mechanisms that control the proper execution of mitosis in eukaryotic cells. The fidelity of these processes is crucial to ensure balanced partitioning of chromosomes between mother and daughter cell, thus providing an essential contribution to genome integrity maintenance in proliferating cell populations. The basic mechanisms underlying cell division, and many key players, are conserved among eukaryotes, but several molecular details of their controls, whose failure can lead to genomic instability and favour cancer development, are still unknown. We use the budding yeast *Saccharomyces cerevisiae* for our studies, since this unicellular organism is widely recognized as a very suitable model system for eukaryotic cell cycle research. We are currently studying a novel pathway that controls the regulation of mitotic entry in response to replication stress independently of the DNA damage checkpoint.