# REPORT

**Workshop :*Trends and Emerging Fields in Molecular Life Sciences***

The workshop brought together Romanian researchers from eleven American and European universities as well as seven Romanian universities and institutes of the Academy.The goal of this workshop was to tackle the challenges posed by the accelerating pace of information accumulation and rapid changes in technology in molecular life sciences. Cutting edge life science research has to deal today with unprecedented wealth of information resulted from the completion of human and some other ~800 genomes. Entirely new emerging fields generically known as 'omics' - genomics, proteomics, glycomics, metabolomics, interactomics - have lately emerged based on the use of hightroughput technologies. This has a huge potential for tackling complex processes relevant in molecular medicine or biotechnology - as for example understanding protein folding in eukariotic systems for accurately producing functional proteins in native state for therapeutic purposes.

A common challenge in the analysis of genomics data is trying to understand the underlying phenomenon in the context of all complex interactions taking place on various signaling pathways. A statistical approach using various models is universally used to identify the most relevant pathways in a given experiment. During the meeting, an important debate of to whether systems biology approach can take over the simple statistics analysis and the future trends in this cutting edge area of life sciences together with the unprecedented impact of this field has been addressed.

What are the folding and degradation pathways of cytoplasmic proteins in eukariotes? Are molecular chaperones absolutely required for protein folding? Can bioinformatics predict the polypeptide fold starting from DNA sequence? Is glycosylation crucial for folding? Trying to answer these questions, the meeting had contributed to a better understanding of the above issues, with an enormous impact on the future development of Romanian science.

**Mircea Ivan(Tufts Univ School of Medicine, USA)** showed that the molecular response to low oxygen is extremely complex and involves a profound rewiring of the transcriptome. Arguably the best characterized components of hypoxia signaling are the heterodimeric hypoxia-inducible factors (HIF). HIFs are composed of an  subunit, which is rapidly degraded under normoxic conditions via the proteasomal pathway, and becomes stable under hypoxic conditions. The critical event for normoxic degradation of the alpha subunit is enzymatic hydroxylation of evolutionary conserved prolines in HIF, which triggers substrate recognition by a ubiquitylation machinery containing the pVHL (von Hippel-Lindau) tumor suppressor. This oxygen sensing process was first described by his postdoctoral work in Dr. William Kaelin's laboratory at the Dana-Farber Cancer Institute (Boston), as well as by Dr. Ratcliffe's group in Oxford. Recently his group established that the arm of HIFs extends beyond the classic (translated) hypoxia inducible genes, specifically inducing a set of microRNAs with roles in cell survival and cancer formation. The current projects focus on a particular hypoxia regulated miR (miR-210),

which is direct target of the hypoxia-inducible factors (HIFs) and was recently associated with adverse prognosis and metastasis in breast cancer. More specifically, they are in the process of identifying miR-210 targets and evaluating their roles in hypoxic cells survival and tumorigenesis using a combination of *in vitro* and *in vivo* models.

**Stefana Petrescu (Institute of Biochemistry, Romanian Academy)** presented the results of her lab on the homeostasy of the endoplasmic reticulum proteins with emphasis on the equilibrium between folding and degradation of proteins. Specific signals determine the maturation and sorting events that allow the ER export of the newly synthesized proteins. Misfolded proteins that do not qualify for secretion are retained and degraded by the ER associated degradation pathways(ERAD). To investigate this process, she has looked into tyrosinase degradation. Tyrosinase is a secretory protein synthesized in the ER and transported through the secretory pathway towards melanosomes, in melanocytes and melanoma cells.This group found that the transmembrane domain (TMD) controls tyrosinase folding by regulating the chaperone recruitment at the translocon. They also shown that soluble tyrosinase lacking its TMD is retained in the ER becoming an ERAD substrate. A number of mutants and tyrosinase chimeras have been then constructed and characterized in terms of ER retention and dislocation in cytoplasm for proteasomal degradation. The data allowed the identification of particular molecular determinants in tyrosinase molecule and specific degradation pathways involving the ER proteins activated by the unfolded protein response.

**Adrian Salic (Harvard Medical School, USA)** talked about the role of chemical biology in exploring cell division and cell-cell signaling. In his lab he developed a method to detect DNA synthesis in proliferating cells, based on the incorporation of 5-ethynyl-2'-deoxyuridine (EdU) and its subsequent detection by a fluorescent azide through a Cu(I)-catalyzed [3 + 2] cycloaddition reaction ("click" chemistry). They demonstrated the use of the method in cultured cells and in the intestine and brain of whole animals.

**Sorin Draghici (Wayne State University, USA)** presented a systems biology approach to pathway analysis. A common challenge in the analysis of genomics data is trying to understand the underlying phenomenon in the context of all complex interactions taking place on various signaling pathways. A statistical approach using various models is universally used to identify the most relevant pathways in a given experiment. In his talk, he showed that despite its general adoption, this statistical analysis is unsatisfactory, and can often provide incorrect results. Using a systems biology approach, Sorin Draghici developed an impact analysis that includes the classical statistics, but also considers other crucial factors such as the magnitude of each gene's expression change, their type and position in the given pathways, their interactions, etc. On several illustrative data sets, the classical analysis produces both false positives and false negatives while the impact analysis provides biologically meaningful results.

**Stefan Szedlacsek (Institute of Biochemistry, Romanian Academy)** reported on the analysis of molecular determinants of prl-3, a protein tyrosine phosphatase involved in cancer metastasis. The special interest for these PTPs in the last years is due to its overexpresssion in different cancer

forms as well as in metastasis of colorectal cancer. A large number of studies have been performed related to this protein; however, only few data are available at present as concerning its molecular determinants.To evaluate whether a C-terminal polybasic sequence represents a nuclear localization signal (NLS) he obtained several truncated and mutant forms of PRL-3 and analyzed their subcellular localization as compared to the wild type form. The results invalidated the hypothesis that this was an NLS.Looking at the influence of the C- and N-terminal residues on the phosphatase activity of PRL-3. they found that the C-terminal CAAX motif, besides directing the protein farnesylation, plays an additional regulatory role by inhibiting the catalytic efficiency of PRL-3.
Accounting for the results we are reporting here, as well as for those reported in literature, Stefan proposes a hypothetical molecular mechanism for the nucleocytoplasmic localization and transfer of PRL-3.

**Andrei Petrescu (Institute of Biochemistry, Romanian Academy)** described the ***in-silico* methods in structural biochemistry.** He presented results obtained in the last couple of years related to bioinformatics and molecular modeling tools and strategies developed in the Department of Bioinformatics and Structural Biochemistry of the Institute of Biochemistry of the Romanian Academy for assisting experimental work in the study of proteins and their post-translational modifications. These techniques are currently used for example - in the frame of Bioexploit FP6 integrated project - in assessing the structure-function relation in proteins recently sequenced that were shown to be involved in plant-pathogen interactions. Examples were also shown from other national and international projects in which molecular modelling results were effective in guiding and assisting molecular cell biology endeavors.

**Dan Duda (Harvard Medical School, USA)** presented his work destined to the understanding of tumor pathophysiology using preclinical studies.Preclinical studies over the last three decades have provided strong support for the use of antiangiogenic therapy as an approach to cancer treatment. Despite setbacks, the clinical development of antiangiogenic agents has accelerated remarkably over the past 3–4 years. Consequently, there are currently three direct inhibitors of the VEGF pathway approved for use in cancer patients. Other agents that block the VEGF pathway are in advanced stages of clinical development and have shown promising results. With these exciting developments come crucial questions regarding the use of these new molecular-targeted agents, alone or in combination with standard cytotoxic or targeted agents. Importantly, the mechanisms of action of anti-VEGF therapy remain unknown. The challenge now is to return to the bench-side to identify in translational studies mechanisms of action and validate biomarkers for antiangiogenic therapy. He further discussed preclinical and clinical studies revealing potential mechanisms such as tumor vascular normalization, bone marrow-derived cell recruitment blockade and cytostatic effects of anti-VEGF therapy. Finally, Dan Duda reviewed the current progress and the future directions for anti-cancer therapy using anti-VEGF agents, emphasizing clarification of the underlying molecular and cellular mechanisms of action and biomarker identification and validation.

**Norica Nichita(Institute of Biochemistry, Romanian Academy)** talked about her work on targeting the host endoplasmic reticulum α– glucosidases as antiviral therapy against

human hepatitis B virus. The mechanism underlying the antiviral effect of an □-glucosidase inhibitor, the iminosugar N-butyldeoxynojirimycin (NB-DNJ), against the human hepatitis B virus (HBV) was investigated. The folding, oligomerization, and assembly of the HBV envelope glycoproteins take place in the endoplasmic reticulum (ER) of the infected cells and depend on the chaperone proteins of the ER-quality control machinery. She analyzed the biosynthesis, maturation, N-glycan processing, of HBV envelope glycoproteins designated S (small), M (middle) and L (large) in the presence and absence of the inhibitor. Secretion of subviral particles from cells treated with the NB-DNJ and infectivity of released virions, were also investigated. Her results show that NB-DNJ treatment results in impaired secretion of the HBV M and L envelope glycoproteins-containing subviral particles, while the S protein is less affected. However, only the M protein is retained within the cells and subsequently degraded. Using the newly developed HepaRG cells permissive for HBV infection *in vitro,* we were able to show that infectivity of HBV released from infected cells is severely affected by NB-DNJ, in dose-dependent manner. Aspects related to a potential use of NB-DNJ as antiviral drug in combination with currently approved drugs, for treatment of chronic HBV infection were also discussed.

**Costin-Ioan Popescu ( CNRS, Institut de Biologie, France)** presented his work on the Hepatitis C virus morphogenesis. This virus represents a global health problem with a prevalence of approx 3% of the whole world population. . 80% of patients are cronically affected and the disease evolves towards hepatic fibrosis and hepatocarcinoma. The present treatment has a limited efficiency and new antiviral strategies are sought. The recent development of a tissue culture system that allows the complet HCV cycle reproduction allows the study of the virus morphogenesis as a therapeutic target. He then presented various strategies to block the assembly process of the viral particle Using biophysical (FRET-FLIM) or biochemical (immunoprecipitation ) techniques we aim to understand the network of protein-protein interactions that generate the virus. The other approach is the identification of endogenous proteins involved in the HCV morphogenesis. Particularly, he is studying the relationship between misfolded proteins and the autophagy in the viral assembly process.

**Octavian Voiculescu (University of College London, UK)** discussed his integrative vision on amniotic gastrulation. Gastrulation generates the basic body plan through massive cell movements and tissue rearrangements. He presented results which identified some of the basic cell behaviours and iteractions underlying this process in a representative amiote (the chick embryo). A model of the gastrula, which is based exclusively on the data from these reductionist approaches.was then presented The usefulness of this model was discussed in terms of ability to model the shaping of the embryo and guide experimental work."

**Ioan Ovidiu Sarbu ( University of Ulm, Germany)** gave a talk on the in vivo signaling using the retinoic acid in mouse embryons. One of the main conclusions of his study is that antagonism of Fgf8 expression by retinoic acid occurs in the ectoderm and that

failure of this mechanism generates excessive FGF8 signalling to adjacent mesoderm, resulting initially in smaller somites and then left-right asymmetry.

**Nicanor Moldovan** (**Ohio State University, USA**)described his work on bone marrow stem cells in the spontaneous repair of stroke. His lab has identified an unexpected spontaneous re-oxigenation of the infarct area based on the proteolytic activity of the inflamatory infiltrate, as well as the formation of oxigen and nitrogen free radicals. Thier intearction with vital cellular proteins limits their survival in the infarct area. He finally discussed the demonstration using an oroginal method of the capacity of stem cells isolated from boine marrow to stimulate angiogensis in vivo and their use in tissue engineering.

**Mihai Ciubotaru** (**Yale University School Medicine, USA**) presented ta biochemical study of the intercations of circular DNA with RSSs, tensioned in various forms of supercoiling with RAG recombinase. He highlighted that the orientation of RSS is critical for catalysis. These mechanisms explain the discrimination of recombinase for intrachromosomial sites. The translocation resulted during fisiological variations has implications in the pathology of B and T lymphoma.

**Alexandru Babes (University of Bucharest, Romania)** reported on the role of sodium channel in cold detection in mammals. Although the visual acuity and motor activity are deeply inhibited by cold temperatures, teh ability for pain perception is unaltered, so that the cold induced pain can become unbearable. The fact that neurons involved in pain stimulus detection (nocireceptors) continue to function at low temperatures is essential for the organism survival and adaptation and is due to the special properties of the channel sodium dependent of voltage and independent of tetrodotoxin (TTX) Nav1.8. This channel is mainly expressed in nocireceptors. Inactivation of the channel Nav1.8 is totally insensitive to cold. Mutant mice with the gene Nav1.8 inactivated showed massively attenuated responses to cold and mechanical stimulation at low temperatures. In conclusion the data showed that Nav1.8 is a specialized molecule for detection of painful cold and other type of painful stimuli at low temperatures.

**Marin Gheorghe (Tyndal National Institute, Ireland)** gave a very interesting talk regarding the microfluidics technology for building microarray devices. These technologies may have an important impact in the development of microarrays analysis of various genes involved in health and patological states.

**Sergiu Fendrihan (University Salzburg, Austria)** presented a new project destined to the generation of a new Bioresources Center in Romania.

**Irinel Popescu ( Surgery and Hepatic Transplant Center, Romania) and Daniel Funeriu** (**Techische Universitat Munchen, Germany**)have chaired an exciting round table regarding the involvement of Diaspora in the research and education in Romania. Discussions revealed that whilst the process of evaluation for grants and for research institutes started to be promissing, there is still much to do in order to establish the best criteria for those purposes.

An interesting analysis of the evolution of the biological research has been presented by **Octavian Popescu ( Babes -Boliay University Cluj, Romania)** who reviewed the scientometric results of the biologists biochemists and cell biologists in Romania.

It is now an exciting time for biology and this special meeting on "*Trends and Emerging Fields in Molecular Life Sciences*" is significant by having focussed on the new insights deriving from the genome projects. This was a forum for exchange of new results, concepts and ideas in the field of protein biochemistry and molecular biology between European and American scientists of Romanian origin. Bringing together biochemists, cell biologists, immunologists, physicians the aim of the meeting was not only to reach a vision for the future development of protein sciences, but also to establish scientific contacts between scientists with common interests. .

This broad discussion could not have been accomplished without the kind invitation of the Academy and financial support by ANCS. Therefore we would like to express our thanks to these two organisations and personally to Professor Ionel Haiduc, President of the Academy, Professor Anton Anton, President of the ANCS, Cati Alexoaiei, Assistant Director CNCSIS and and Luciana Bratu, International Projects Coordinator at CNCSIS for all their interest and help.

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