Triplet

The Quarterly of the Faculty of Biochemistry, Biophysics and Biotechnology, JU

A special edition 1 /2011

A special edition devoted to the 'Molecular Biotechnology for Health' project

"Molecular Biotechnology for Health"

The "Molecular Biotechnology for Health" (MBH) is a project realised at the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology. The allocated grant is 28,197,842 PLN and is financed by the European Union funds (the European Regional Development Fund, the Innovative Economy Operational Programme for 2007-2013). The project runs from September 2008.

Present undertakings comprise seven investment and research tasks coordinated by the employees of the Faculty. One of the main aims of the project is to establish the following laboratories:

- The Animal Facility,
- The Laboratory of Imaging Cytometry,
- The Laboratory of Proteomics and Transcriptomics,
- The Laboratory of Cell and Tissue Engineering,
- The Laboratory of Viral Molecular Diagnostics,
- The Laboratory of Plant Biotechnology,
- The Biological Sample Storage System.

This project contributes to improving the Faculty's research infrastructure. World class equipment enables researchers to implement new experimental techniques, which are:

- the development and application of new animal disease models and the assessment of experimental therapies and treatments,
- the elaboration of new methods of cell structures analysis and the interaction of studied substances with cells,
- the development of a complex system of gene expression analysis at the transcriptome and proteome level,
- the development of skin tissue cultures with the aim of its application in clinical conditions,
- the elaboration and development of methods allowing for the identification and quantification of human and animal viruses in relation to their application in clinical and research conditions,
- the identification, study and elaboration of plant metabolite derivatives as well as photosynthetic dyes derivatives for use as potential anti-tumour drugs.

In this special issue of "Triplet", prof. Alicja Józkowicz and prof. Halina Gabryś write about the tasks that they coordinate within the framework of the MBH project and which are respectively: the organization of the modern animal facility and the laboratory of plant biotechnology . These were two tasks with which the project coordination team was occupied the most during the first and second quarters of 2011. Additionally, the coordinators of the other tasks relate about what is currently happening in their laboratories. We also write about the grants which are being realised by the Faculty's employees using scientific equipment purchased from "Molecular Biotechnology for Health" funds.

We hope you enjoy this special issue of the quarterly. We also invite you to visit the project's website: http://bmz.wbbib.uj.edu.pl/

Magdalena Jagła

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INNOVATIVE ECONOMY
NATIONAL COHESION STRATEGY

The Molecular Biotechnology for Health project (POIG.02.01.00-12-064/08) is financed by the European Union from the European Regional Development Fund within the framework of the Innovative Economy Operational Programme for 2007-2013.

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND

THE ANIMAL FACILITY

The animal facility before adaptation

Corridor in the animal facility

The breeding unit

The experimental unit

Construction of the new animal facility concluded in April. The last remaining construction elements, which are the flow sterilizers, an automatic wash and a laminar flow cabinet with an access window, were also delivered. The site acceptance testing, which included obtaining approval from the Chief Sanitary Inspectorate and all of the other required control authorities, took place in early May. The last stage will involve the purchase of the cages and is planned for the third quarter of 2011. The first animals will be introduced to the facility in autumn.

Eventually, the animal facility will house about 8000 mice and 250 rats. It will also be able to house about 20 rabbits and 100 gerbils or hamsters. The animals will have to come from certified breeders and to possess a health certificate. Commercial strains which were unavailable before introduction into the animal facility will have to be renewed through embryo transfer techniques. This requirement is necessary to maintain the sanitary regime which will ensure that scientists obtain reproducible results, especially in immune system research. The experiments will be carried out by scientists and graduate students in charge of the various projects. Day-to-day maintenance and animal care, on the other hand, will be provided by the staff of the facility.

The facility will consist of three zones:

- a breeding zone which will only be accessed by authorized personnel and where unique mouse and rat strains will be bred,
- an experimental facility with broader access and where the laboratory animals will be housed and where surgical procedures and vital analysis will be carried out,
- a technical zone including animal feed and litter storage, feeding and sterilizing facilities.

The entire animal facility will use the Individually Ventilated Cages (IVC) system which will ensure sterile conditions for the animals including immunodeficient strains. There will also be a cage exchange station, litter removal station and fume hoods enabling researchers to carry out all necessary actions related to the breeding and housing of the animals in sterile conditions to ensure the safety of both the employees and the animals. The only place where the animals will leave the sterile cages will be the surgical and in-vivo analysis rooms. Both of these rooms are equipped with UV lamps and high-throughput

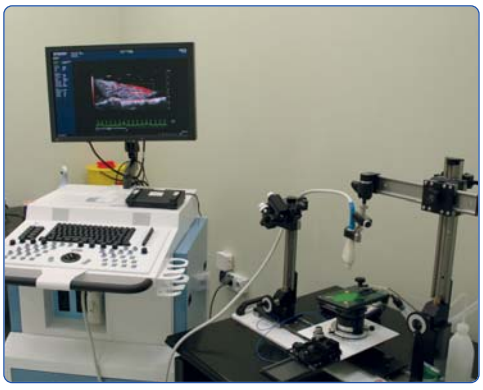
HEPA filters in both the incoming and outgoing ventilation tracts what guarantees sterile conditions. The facility will also contain anaesthesia, euthanasia and organ perfusion stations equipped with fume extraction systems. Currently it will be the only such an animal facility in Poland.

The facility will include a surgical room equipped with an anaesthesiology set, a heated surgical table and smaller tables, a surgical microscope, surgical tools, a veterinary haematological and biochemical analyser, a laminar flow cabinet, a CO_2 incubator, a microscope and equipment necessary for tissue isolation and the creation of cell lines. There will also be a Langendorff system for the study of isolated rodent hearts. Therefore, it will be possible to monitor the health of the animals, to carry out all types of microsurgical procedures on mice and rats and to collect tissue samples and create primary cell lines. The cultures will either be continued in the animal facility or transferred to other laboratories for further analysis.

The analysis room will be equipped with a special Vevo-2100 high-resolution ultrasonograph, a system for in-vivo luminescence and fluorescence detection and a Doppler flow cytometer. This equipment will be used for various research aims, including the study of tumour growth and metastasis, the monitoring of cardiac and large blood vessel function, study of arteriosclerosis development, tissue perfusion levels, organ structure, embryo development, stem cell research etc. This will allow all of the Faculty's groups that work on animal models to carry out a diverse range of experiments and to collaborate with other experimental institutions as well as pharmaceutical or biotechnology companies. We hope to see you there!

Prof. Alicja Józkowicz

Autoclave



Ultrasonograph Vevo-2100

Site acceptance testing

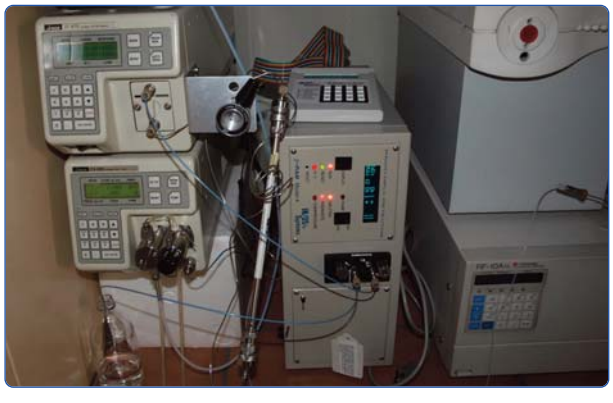
Team participating in the investment

THE LABORATORY OF PLANT BIOTECHNOLOGY

Laboratory of Plant Biotechnology

Gas chromatograph radioactivity detector

Multispectral Imaging System



Radioactivity detector

The members of the Department of Plant Biotechnology have created two professionally equipped laboratories for the molecular study of plant nucleic acids and proteins with funds from the "Molecular Biotechnology for Health" project. In addition to basic equipment required for molecular biology, they also acquired a gene gun (Helios Gene Gun System BioRad) and an image documenting system (BioSpectrum, UVP). The gene gun is used mainly for biolistic transient transformation of plant cells, and the BioSpectrum system is used for analysis of experiments using chemiluminescent and fluorescent dyes and marker proteins. It is used in particular to analyse polyacrylamide gels on which plant proteins are separated via two-dimensional electrophoresis as well as for all types of *Western blotting* analysis.

The work done in the Department of Plant Biotechnology has two main aims. The first is the study of light signal transduction mechanisms and the cross-talk of signal transduction pathways initiated by various stimuli in higher plants. The second is the investigation of DNA repair and replication mechanisms with a special focus on the role of the PCNA protein in these processes.

PCNA (Proliferating Cell Nuclear Antigen) is a fundamental protein of all eukaryotic organisms. The lack of this protein in the initial stages of development invariably leads to death. Among other things, this protein is involved in the regulation of the cellular cycle, DNA genome replication and DNA repair processes. Although PCNA plays an essential role in eukaryotic organisms, not much is known about its function in plants, in particular, about the role it plays in the regulation of the cell cycle. The aim of our research is to elucidate which of the plant proteins responsible for cell cycle regulation interact with PCNA. To achieve this, the team is employing the BiFC (Bimolecular Fluorescence Complementation) method using transient transformation. Constructs are used for the transformation, in which the investigated genes are fused with genes coding for half of the report protein, GFP. The appearance of fluorescence indicates that an interaction between proteins has occurred.

At the moment, studies on the impact of light and plant age on the expression of the genes coding for blue light photoreceptors – phototropins have been concluded. The aim of these studies was to determine the influence of other photoreceptors active in plants, i.e. phytochromes and cryptochromes and of the phototropins themselves on the levels of phototropin mRNAs. One of the methods used in these experiments was quantitative real-time RT-PCR. A similar cycle of studies was carried out for genes encoding two plant chlorophyllases, enzymes participating in the catabolism of chlorophyll which are especially active during times of stress and plant aging. The team was able to show for the first time that the levels of mRNA of these genes are regulated by light.

Another goal of research is to determine the role of phototropins in the regulation of flowering in *Arabidopsis*. The results obtained thus far indicate that phototropin 1 plays a novel role in the photoperiodic control of flowering. The method of transient

transformation is also used to probe the activity of signal proteins in plant cells subjected to different combinations of light wavelengths. In particular, the team is monitoring the activity of phospholipase C, an enzyme of the phosphoinositide signalling pathway, using a biosensor GFP-PH: the δ1 domain of phospholipase C which binds PIP2 (phosphatidylinositol 4,5-biphosphate), fused to GFP. The observation (in a confocal microscope) of GFP fluorescence resulting from transient expression of the biosensor in the leaf cells of *Nicotiana benthamiana* provides information about changes in the enzyme activity.

The team is also conducting novel research on the SUMOylation of PCNA and phototropins. SUMOs (small ubiquitin-related modifiers) are ubiquitin-like proteins which can reversibly modify post-translationally various target proteins. The experimental model being used is the *Arabidospis* SUMOylation pathway reconstructed in *E. coli*. The team investigates which SUMO isoforms are responsible for the modification of the studied proteins. Furthermore, the team determines the potential target residues using, among other techniques, site directed mutagenesis of lysines potentially recognized by SUMOs. There are also experiments planned to determine the physiological role of this modification in the function of the studied proteins.

This research has and is being funded by several Polish and European grants:

- the Polish Ministry of Science and Higher Education grants "Impact of light on the actomyosin system active in chloroplast movements" and "Factors involved in the regulation of chlorophyllase expression and activity" which concluded in 2010;
- projects currently being carried out: i) ITN UE 7 Framework Programme "Chloroplast Signals" (COSI) and the accompanying grant "Signals from chloroplasts" - the role of calcium and of the phosphatidylinositol pathway in chloroplast response to light stimuli, ii) Polish Ministry of Science and Higher Education grant "Molecular analysis of *Arabidopsis thaliana* PCNA" - characterization of post-translational modification of AtPCNA proteins (including SUMOylation and ubiquitination), analysis of the influence of mutagenic factors on the expression of these genes, assessment of the impact of AtPCNA gene knock-out in the process of DNA repair and replication, iii) Iuventus Plus (Polish Ministry of Science and Higher Education grant) "Analysis of interaction networks between PCNA plant protein and selected proteins involved in the regulation of the cell cycle in plants" – includes the identification of proteins which interact with PCNA using the BiFC method;
- iv) the Polish National Centre for Research and Development LIDER programme, starting in November - the search for PCNA inhibitors which may have a potential application in anti-tumour therapy.

Prof. Halina Gabryś

Spectrophotometer

Plant transformation with the gene gun

Spectrophotometer

Horizontal autoclave

THE LABORATORY OF IMAGING CYTOMETRY

Confocal microscope Leica SMD (FLIM, FCS)

Studies of the cellular nucleus and of the extracellular matrix are being carried out in the Imaging Cytometry Unit using the Leica SP5/STED and Leica SMD (FLIM/FCS) confocal microscopes.

There is on-going work being carried out on optimizing methods for inducing local chromatin damage with visible wavelength beams and without the use of photosensitizers. This method will serve to study the recruitment of repair proteins involved in DNA oxidative stress damage and strand break repair processes. We are investigating the photoconversion of DNA-binding fluorescent stains. The goal of these studies is to use the photoconversion of fluorescent stains to develop a new method for imaging oxidative DNA damage with modern fluorescence microscopy methods.

Studies are also being continued into the mechanisms of DNA damage induced by drugs such as camptotecin, topotecan and mitoxantrone, by ultraviolet radiation and by hydrogen peroxide. These experiments focus on the damage arising in the region of the DNA replication forks. Work is being continued on the role of heterochromatin protein 1 in different chromatin damage repair processes, including the repair of oxidative stress damage and of single and double strand DNA breaks. Studies of the binding mechanisms of Pyronin Y to DNA and RNA have been concluded. These included studies of fluorescence quenching of RNA-bound Pyronin Y in fixed cell nucleoli. Studies of the interactions between drugs with DNA-affinity and chromatin in live cells are also on-going. The structural changes of chromatin induced by minor-groove binding and nucleotide intercalating agents are being investigated. A technique for the detection and visualization of single fluorescent molecules will also be used within the scope of this project.

Studies probing the dynamics of the H1 linker histone in different phases of the cell cycle and in drug-treated cells have begun. We are also investigating the structure of the extracellular matrix using the Col-F fluorescent stain to study vascular wall collagen.

Prof. Jerzy Dobrucki

THE LABORATORY OF PROTEOMICS AND TRANSCRIPTOMICS

Instrument for real-time analysis of macromolecular interactions

The following are the main fields of research carried out in the Laboratory of Proteomics and Transcriptomics during the period leading up to June 2011.

In a collaboration between the Departments of Analytical Biochemistry and Plant Biotechnology (Dr. Wojciech Strzałka), the surface plasmon resonance (SPR) measurements on a Biacore 3000 system were applied to complete the comparative characterization of the binding of the AtPCNA protein (*Arabidopsis thaliana* Proliferating Cell Nuclear Antigen) and it's mutant to a fragment of At-SCE (a SUMO conjugating enzyme). This intereaction determines the functionality of the protein's SUMOylation process. A manuscript containing these results has already been submitted for publication. In a collaboration with the Department of Microbiology (Grzegorz Dubin, Ph.D.), an attempt was made to characterize the binding of active centre peptides derived from the protein p53 and its analogues to a wild type and a mutant of the Mdm protein. A continuous research is carried out on the macromolecular interactions within the systems that generate bioactive pro-inflammatory peptides called kinins. This topic is one of the main areas of interest in the Department of Analytical Biochemistry (Maria Rąpała-Kozik, Ph.D., Marta Kujda). Recent experiments allowed to determine the kinetic and thermodynamic constants of reactions between two contact system proteins, the high-molecular weight

kininogen (HK) and prekallikrein, as well as to identify and initially characterize the binding of myeloperoxidase to HK. The newest developments in this area are studies carried out in a collaboration with the Department of Physical Biochemistry (Andrzej Górecki, Ph.D., Filip Gołębiowski) aimed at elucidating the influence of DNA activation, inhibition and initiation sequences on the structural changes in specific domains of the transcription factor Yin Yang 1.

The Bruker HCT ultra mass spectrometer (an ion trap equipped with an ESI ion source) has been used for fundamental analyses in the areas of protein and peptide chemistry performed in the Department of Analytical Biochemistry. The most recent studies focused on the fragmentation of kininogens and kinin generation by Sap2, the main secreted proteinase of the pathogenic yeast *Candida albicans* (Oliwia Bocheńska, Grażyna Braś). A draft of the publication containing the results of these experiments is currently in preparation. This same method was also used to identify some antibacterial peptides (Paweł Mak, Ph.D. D.Sc., Department of Analytical Biochemistry) and as a part of a routine characterization of chlorophyll derivatives synthesized in the Department of Plant Physiology and Biochemistry (Leszek Fiedor, Ph.D. D.Sc.). There is also on-going collaboration with the Department of Developmental Plant Physiology and Biology (Dariusz Dziga, Ph.D.) to characterize cyanobacteria toxins and with the Department of Microbiology (Tomasz Kantyka, Ph.D.) to analyse the citrullination of antibacterial peptides.

The Leica LMD 7000 Laser Microdissection System is being used to analyse the cellular structures cutted out of frozen and paraffin samples. One of the projects being carried out at this moment is an attempt to determine the phenotype of stem cells transplanted into the region of cardiac muscle after heart infarction in pigs (these studies are being carried out by Prof. Józef Dulak, Prof. Alicja Józkowicz and Krzysztof Szade from the Department of Medical Biotechnology with the collaboration of Prof. Michał Tendera and Wojciech Wojakowski, Ph.D. from the Medical University of Silesia). Furthermore, Agnieszka Łoboda, Ph.D. is working with Prof. Elżbieta Pyza and Milena Damulewicz from the Department of Cell Biology and Imaging in the Zoology Institute, Faculty of Biology and Earth Sciences, to isolate structures from *Drosophila melanogaster* visual system. These studies aim to analyse the gene expression profile responsible for regulating fly's circadian rythm.

Prof. Andrzej Kozik, Agnieszka Łoboda, Ph.D.

Ultra centrifuge Sorvall WX Ultra Series

Laser Microdissection System

THE LABORATORY OF CELL AND TISSUE ENGINEERING

There are currently four research projects being carried out in the Laboratory of Cell and Tissue Engineering. Two grants are from the Polish Ministry of Science and Higher Education entitled :

- "Clinical application of autologous human skin cells in combination with *Integra DRT* dermal scaffold for the treatment of burns – an analysis of regenerative processes based on an in-vitro model."
- "MCPIP protein in the regulation of keratinocyte differentiation and inflammatory processes".

The other two projects involve structural funds from the following European Union Grants:

- "Innovative methods of stem cell application in medicine – Investigation of the role and biological properties of VSEL cells in skin regeneration".
- "Application of polyisoprenoid derivatives as drug carriers and metabolism regulators".

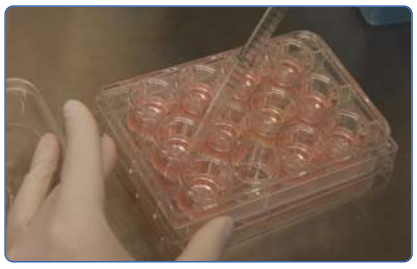
In-vitro cultured human skin cells are used in these projects both for basic research and for clinical trials.

Justyna Drukała, Ph.D.

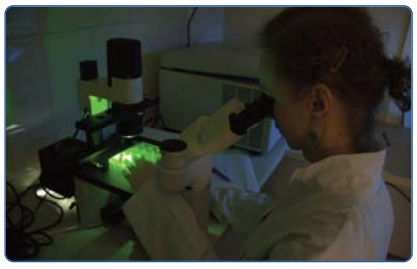
The Laboratory of Cell and Tissue Engineering

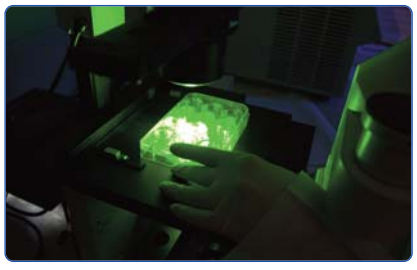
CO_2 incubator

THE LABORATORY OF VIRAL MOLECULAR DIAGNOSTICS



Cell culture dishes





Fluorescent microscopy analysis of viral infections

The "Molecular Biotechnology for Health" project has permitted us to create a fully-equipped laboratory of virology. Work here focuses on questions regarding human respiratory tract infections and on the development of novel diagnostic methods. The studies being carried out at this moment focus on various aspects of the mechanisms and pathogenesis of viral infections.

Viral infection in the context of bacterial infections. We are studying the effect of viral infections on subsequent bacterial infections (e.g. bacterial adhesion, the production of pro- and anti-inflammatory factors, the modification of the expression profiles of surface proteins in infected cells) as well as the impact of bacterial infections on posterior viral infections (e.g. the proteolytic activation of fusion proteins). Studies are being carried out using a culture of fully differentiated respiratory tract epithelium. This model is unique, as it allows for the analysis of pathogens in their natural environment. Currently, we are the only group in Poland using such a model and one of only a few such groups in the world.

Development of novel diagnostic methods. This area of research aims to develop new methods for the identification of known viral pathogens attacking the human respiratory tracts through the use of, among others, real-time PCR and isothermal PCR with polymerase carrying helicase activity. Moreover, studies are being carried out in collaboration with many other groups both in Poland and abroad, to identify new viral pathogens. Methods optimised or developed in our laboratory, such as VIDISCA and HexaPrime, are being used in this case.

Mechanisms of human coronavirus infections. These experiments aim to identify the mechanisms through which viruses enter eukaryotic cells. There is an ongoing research on the expression system for viral proteins responsible for receptor recognition and fusion with the cellular membrane. Successful expression of viral proteins in e.g. yeast or insect cells based system would enable us to identify the proteins responsible for the early stages of infection as well as to identify the cellular receptors for human coronaviruses.

Generation of a new oncolytic vector. A new focus of research has commenced within the framework of the Polish National Centre for Research and Development's LIDER programme. The aim is to create a viral vector which will allow safer and more effective anticancer therapy.

Furthermore, there are two projects being carried out that are financed by the Polish Ministry of Science and Higher Education (the Iuventus Plus programme entitled "Mechanisms of HCoV-NL63 and HCoV-HKU1 virus infections" and research grant entitled "HexaPrime: a new method for identification of RNA viruses") as well as a project financed by the Foundation for Polish Science (Homing programme grant entitled "Influence of extracellular bacterial proteases on viral infection").

Krzysztof Pyrć, Ph.D.

THE BIOLOGICAL SAMPLE STORAGE SYSTEM

Containers for liquid nitrogen

A modern cell bank has been created within the framework of the "Biotechnology for Health" project. It will be used to store patients' cells which will then be used for burn therapy. The human cells are cultured in the Laboratory of Cell and Tissue Engineering of the Faculty of Biochemistry, Biophysics and Biotechnology. These cells are used both for basic research and clinical trials.

Research grants realised with the use of the equipment purchased within the project "Molecular Biotechnology for Health"

Agnieszka Łoboda, Ph.D., from the Department of Medical Biotechnology has received a PARENT-BRIDGE Programme grant from the Foundation for Polish Science for a project entitled "Significance of apolipoprotein E polymorphism in regenerative medicine". The project will be financed for three years with an award totalling 560,000 PLN. In this project, Dr. Łoboda and the research group will focus on defining the role of apolipoprotein E (ApoE) polymorphism in the wound healing process, with particular emphasis on the role of miRNAs and endothelial progenitor cells.

A range of various methods will be applied during this project (e.g. isolation of endothelial progenitor cells (EPCs) and primary keratinocytes, the identification of specific miRNAs in wounds), and modern equipment will be used, including instruments purchased with "Molecular Biotechnology for Health" project funds. One of these is a laser microdissection system (Leica Laser Microdissection LMD7000), which will be used for the analysis of skin sections (healthy and injured) and for cutting individual cellular structures from the skin followed by the determination of gene expression.

This project will be implemented with international collaboration. Optimization of the laser microdissection method will be performed in cooperation with Prof. Grietje Molema (Department of Pathology and Medical Biology, University Medical Centre Groningen, The Netherlands) and the characteristics of EPCs in hypoxic conditions will be studied in collaboration with Prof. Lorenz Poellinger (Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden).

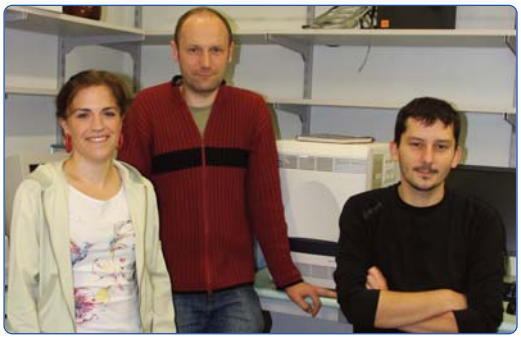
Aneta Kasza, Ph.D. from the Department of Cell Biochemistry has received a PARENT-BRIDGE Programme grant from the Foundation for Polish Science for a project entitled "Regulation of proteolytic activity in the central nervous system". The project will be financed for three years with an award totalling 686,000 PLN. The aim of the project is to explore the mechanisms responsible for half-life regulation of the transcripts of proteins engaged in the regulation o proteolytic activity in the central nervous system.

An Applied Biosystem 7500 Real-Time PCR System and Biological Sample Storage System will be used in this project (instruments purchased with funds from the project "Molecular Biotechnology for Health").

The research will be performed in collaboration with Prof. Tomasz Kordula form Virginia Commonwealth University, School of Medicine, USA.

Agnieszka Łoboda, Ph.D.

In 2010, **Grzegorz Dubin**, Ph.D. of the Department of Microbiology received a LIDER programme grant from the Polish National Centre for Research and Development (NCBiR). The funding encompasses implementation of the project entitled "Anticancer therapy of the future – a search for low molecular weight activators of the p53 pathway". For this project NCBiR will finance the costs of research and employment of scientists for a period of three years with an award totalling one million PLN. The project involves a search for low molecular weight activators of the p53 pathway able to bind and inhibit the function of its main negative regulators whose overexpression is documented in a number of tumour cells.



Grzegorz Dubin, Ph.D. (center), with his team

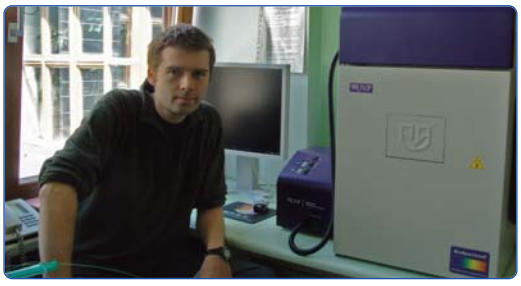
Due to the large diversity of proliferative diseases, the most valuable targets for the development of new therapies are the common elements for a number of cancers. The p53 pathway constitutes one such universal pathway. Inactivation of p53 is observed in the majority of tumour cells. It was demonstrated in

animal models and in preliminary clinical studies that restoring the functionality of this pathway by targeting the overexpressed negative p53 regulators results in tumour regression. Thus, the project uses modern, high-throughput methods of ligand selection to identify molecules capable of reactivating the p53 pathway in cancer cells. The activity of selected molecules is evaluated in a variety of laboratory models to select the best candidates for further development. Access to modern equipment available through, among others, the project "Molecular Biotechnology for Health" (MBH) is of great benefit to the progress of the project. At present real-time PCR equipment and termocyclers obtained from MBH funds are being used in the project. Access to the Biacore system is also provided which will contribute to the detailed characterization of the interactions between the developed molecules and their target proteins.

Magnetic cell sorter

"Molecular Biotechnology for Heath" funds were used to purchase a MACS automatic magnetic sorter for **the Department of Immunology**. This equipment is being used for the sorting of immune cell subsets from human peripheral blood, including plasmacytoid dendritic cells (pDCs), a unique cell type of the DC lineage that differ markedly in morphology from the classic DC archetype (such as myeloid DCs). Despite comprising less than 1% of peripheral blood mononuclear cells in healthy human individuals, pDCs appear to be central to antiviral immunity and autoimmunity. Research on pDCs is supported by a TEAM/2010-5 grant from the Foundation for Polish Science awarded to Joanna Cichy, Ph.D. D.Sc. More information about the TEAM project can be found at http://chemeryna.wbbib.uj.edu.pl/

In recent years, **Wojciech Strzałka's**, Ph.D., research has focused on the study of plant PCNA (proliferating cell nuclear antigen) protein. Initially, his studies focused on the identification of PCNA-like genes, followed by an analysis of the protein properties encoded by these genes and their roles. Afterwards, funds from the Canon Foundation in Europe made it possible for him to carry out research at Osaka University which resulted in the first ever solution of the three dimensional structure of PCNA1 and PCNA2 proteins from *Arabidopsis thaliana*. The



Wojciech Strzałka, Ph.D.

detailed structure of AtPCNA is a starting point for investigating the reasons for their distinct role in DNA repair, which has been demonstrated in recent years.

In addition to research on the role of PCNA in plant cells, Strzałka has also been recently interested in the practical use of the PCNA protein as a target for anticancer therapy. The LIDER program grant has provided him with the opportunity to create a research group searching for low molecular weight inhibitors of the human PCNA protein. Selected molecules will later be tested as possible candidates for a universal and effective anticancer drug. Participation of PCNA in many different processes including DNA replication, DNA repair and cell cycle regulation makes it an essential factor for any dividing cell including cancer cells. Taking into account the multifunctional role of PCNA, this protein appears to be an attractive and universal target of anticancer therapy. The expected final result of the proposed project will be a set of selected inhibitors with high affinity to specific regions of the PCNA protein. The LIDER project will be carried out in the Department of Plant Biotechnology of the Faculty of Biochemistry, Biophysics and Biotechnology and is the beneficiary of structural funds from the European Union's "Molecular Biotechnology for the Health" (MBH) project. The advanced laboratory equipment financed by MBH will be used in the development of PCNA inhibitors.

The results obtained from the LIDER project may lead to the practical use of PCNA inhibitors in anticancer therapy. Potential therapeutic application of developed molecules may help to increase both the length and quality of life of patients suffering from cancer. Hopefully, the effectiveness of anticancer therapy will be enhanced in cells with impaired PCNA function. Modern therapeutic treatment using PCNA inhibitors may present a better choice for some types of cancer cells.

Krzysztof Pyrć, Ph.D. from the Department of Microbiology received a LIDER programme grant from the Polish National Centre for Research and Development (NCBiR) for a project entitled "Cancer therapy: generation of a novel oncolytic viral vector". The project will be carried out over three years with an awardtotaling one milion PLN.

Oncolytic therapy is based on the use of modified viruses that can only replicate in tumour cells thus leading to the destruction of these cells and cancer regression. This project will result in the creation of a novel viral vector for use in cancer patients, in particular those suffering from non-small cell lung carcinoma.

This project can be carried out due to the formation of the laboratory of virology and purchase of equipment (including a flow cytometer, a real time PCR system, a class II laminar flow biological safety cabinet, a CO_2 incubator, an inverted fluorescence microscope, a laboratory small-volume microcentrifuge, four independent PCR thermocycles, an electrophoresis system, two special cabinets for working with nucleic acids, two double-chamber refrigerator-freezers (+4/-20°C), a thermomixer, a magnetic stirrer, a computer with a multi-function device) financed from "Molecular Biotechnology for Health" project funds. This modern laboratory equipment will accelerate the research and allow to obtain more reliable results and to work in a safe environment.

An important part of this project will also be carried out with the collaboration of international partners including Prof. Ralph Baric's and Amy Sims', Ph.D., group from the University of North Carolina, whose experience is essential to the successful outcome of the planned research.

Prof. Halina Gabryś, head of the Plant Biotechnology Department, was awarded an EU fund grant within the framework of FP 7, Marie Curie Initial Training Network, to carry out a part of the "Chloroplast Signals" project (COSI, http://www.univie.ac.at/cosi/) coordinated by Vienna University. The project is aimed at the identification of regulatory networks which control chloroplast function higher plant, algae and diatome cells. The research task of the Krakow group is to characterize the network of light-induced signals which control directional movements of chloroplasts in mesophyll cells of land plants, with a special focus on the roles

Krzysztof Pyrć, Ph.D.

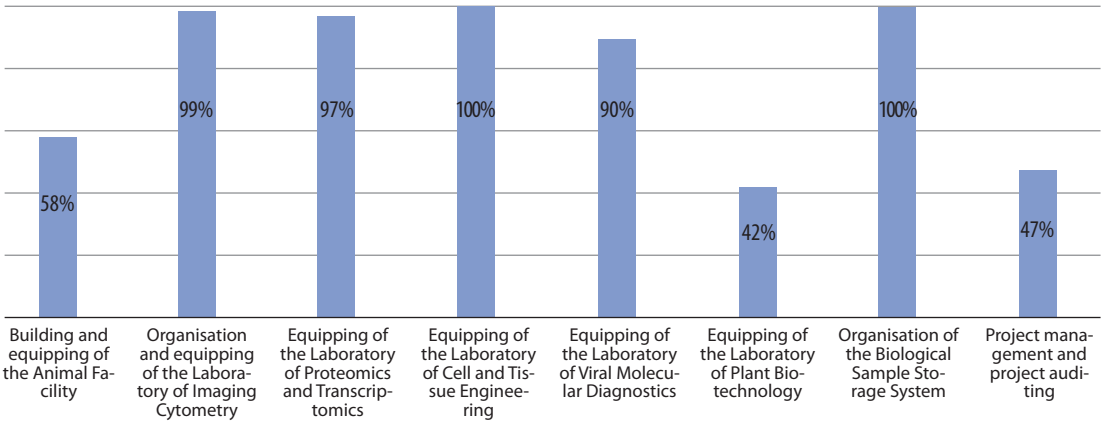
of calcium ions and the phosphoinositide pathway.

The 4-year-long project started in July 2008. The Plant Biotechnology group was awarded 162,000 Euro and 534,000 PLN (the latter is a co-financing from the Polish Ministry of Science and Higher Education). Resources obtained through the "Molecular Biotechnology for Health" (MBH) project have been used to fully equip two laboratories for molecular studies of plants, one focused on nucleic acids and the other on proteins. This has enabled us to extend the work with transgenic plants, an indispensable tool for elucidating the mechanisms of signal transduction in cells. A system for gel analysis and documentation is one of the important devices purchased with the MBH grant. It allows for the imaging of one- and two-dimensional gels stained with pigments detectable in the visible region and with various fluorescent pigments, as well as recording of chemiluminescent signals which is particularly useful in blotting techniques. Moreover, we purchased a gene gun, indispensable for carrying out transient transformation in plants. Gathering information is much faster with this technique as compared to the stable transformation method which requires long-lasting selection and breeding procedures. The investigations are carried out within a consortium which groups ten European partners from both academia and industry. In particular, our research team cooperates with groups of Prof. Ute Vothknecht from the University of Munich, Associate Prof. Markus Teige from Biocenter at the University of Vienna, Prof. Eva-Mari Aro from the University of Turku and Matthew Hannah, Ph.D., from Bayer BioScience in Gent. The aim of the collaborative work is to assess transient changes in calcium concentrations in the cell with the use of transgenic *Arabidopsis* plants expressing a calcium marker, aequorin, in various cell compartments. Further studies are planned using phosphoproteomic techniques.

Gene gun

A summing up of project cost

Up until July 2011 21 625 612 PLN had been spent within the framework of the project i.e. 75% of the total grant given. The percentage of funds spent in relation to the relevant tasks is presented in the graph.



Task nos.	Task name	Planned sum (PLN)	Amount spent (PLN)
1	Building and equipping of the Animal Facility	7 109 984,00	4 136 611,33
2	Organisation and equipping of the Laboratory of Imaging Cytometry	7 104 829,00	7 003 296,00
3	Equipping of the Laboratory of Proteomics and Transcriptomics	5 344 666,00	5 174 276,59
4	Equipping of the Laboratory of Cell and Tissue Engineering	858 304,00	860 003,87
5	Equipping of the Laboratory of Viral Molecular Diagnostics	1 038 259,00	931 266,45
6	Equipping of the Laboratory of Plant Biotechnology	5 013 189,00	2 127 036,15
7	Organisation of the Biological Sample Storage System	562 298,00	550 920,96
8	Project management and project auditing	1 799 228,00	842 201,31
	TOTAL	28 830 757,00	21 625 612,66

In the table is presented the degree to which tasks have been realised with regard to the planned and actual expenditure to date.

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