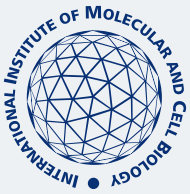
Annual Report 2012

INTERNATIONAL INSTITUTE
OF MOLECULAR AND CELL BIOLOGY



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Directors' Note

For years we have used this space as an opportunity to recall and describe – usually with great pride – our purchases of new research equipment, new grants or successive awards and honours received by our researchers. This positive trend has continued in the IIMCB for some time and, hopefully, it will go on into the years to come. This year we would like to highlight a new development in the life of our Institute: competitions for the positions of lab leaders, run jointly with other institutions.

In fact, this approach has already been tested and it is not completely new. In our 12-year cooperation with the Max Planck Society (Max-Planck-Gesellschaft – MPG) we have been able to develop a model of international cooperation which resulted in the establishment in Dresden and Warsaw of research laboratories on a par with the best research facilities. For our Institute this model of cooperation represented the first and fundamental step towards taking our operations to truly international levels. Continuing this course of action, we decided – again jointly with the MPG – to fill two independent positions: one in the MPI-LHR in Bad Nauheim, the other one in the IIMCB. This model of cooperation is aimed at bringing us closer to yet another partner representing one of the excellent MPG research institutes in Germany. We also advertised a competition, on a slightly different basis, acting jointly with the Institute of Zoology PAN in Warsaw, for an independent research position whose purpose is to lead to the establishment of a genomics laboratory at the PAN Institute co-financed by both institutions. As we know from our experience to date, people are the most important success factor in enterprises of this kind. So far we have been successful in attracting bright young scholars to the IIMCB and in both these cases we hope to be able again to recruit people whose academic vision will enable them to step beyond the old routines.

The first of the research positions described here is related to a very important event in the life of our Institute: the launch of a core facility devoted exclusively to the maintenance and breeding of zebrafish. We have selected this animal model as the optimum choice for all our laboratories that need relevant *in vivo* models for their research. This small omnivorous freshwater fish, whose natural habitat is in the streams flowing off the Himalayas, has become one of the most valued model organisms used for research into development processes, cancerogenesis, issues in the field of genetics, neurobiology, teratology, regenerative medicine, etc. The zebrafish genome has been fully sequenced, the embryonic development of the fish is very rapid and its eggs are large and transparent and they develop outside the mother's body. Most importantly, pathologies modelled in zebrafish are very close to the mammalian and human models. The IIMCB will be the first scientific institution in Poland using this model on such a scale. This has been made possible thanks to IIMCB's winning the *FishMed* project (RegPot, FP7). It is worth emphasising that this high-budget FP7 project is a continuation of projects previously carried out at the IIMCB: *Centre of Excellence* (FP5) and *HealthProt* (RegPot, FP7), both of which based their philosophy on increasing the academic potential of our scientists and stimulating their interaction with leading research facilities worldwide. Together with six other FP7 grants, including three ERC grants, and 21 domestic grants (NCN, NCBiR), and with an average success rate exceeding 70%, this shows a very favourable image of the extramural fundraising potential of the IIMCB, both domestically and abroad.

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Directors and International Advisory Board of the International Institute of Molecular and Cell Biology

2010-2014 term

Jacek Kuźnicki
Director

Michał Witt
Deputy Scientific Director

Jacek Jaworski
Deputy Director

Hanna Iwaniukowicz
Financial Manager

From left (first row): F. van Leuven, J. Kuźnicki (non-member), R.P. Erickson, H. Saibil, A. Tramontano, A. Wlodawer, M.J. Nałęcz, W. Filipowicz; (second row): N. Blin, I. Braakman, M. Witt (non--member), K. Hahlbrock, A. Azzi, J. Mallet, D. Picard.

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Ineke Braakman. Utrecht University, Utrecht, Netherlands
Ivan Dikič. Institute of Biochemistry II, Goethe University Medical School, Frankfurt am Main, Germany
Robert P. Erickson. University of Arizona, Health Sciences Center, Tucson, USA
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Didier Picard. University of Geneva, Sciences III, Geneve, Switzerland
Helen Saibil. Birkbeck College London, Institute for Structural and Molecular Biology, London, UK
J. Gregor Sutcliffe. The Scripps Research Institute, La Jolla, California, USA
Adam Szewczyk. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
Anna Tramontano. University of Rome "La Sapienza", Rome, Italy
Alexander Wlodawer. National Cancer Institute, Frederick, USA

Description of the Institute's Activities

Brief history and principles of activity

The International Institute of Molecular and Cell Biology in Warsaw (IIMCB) is one of the most modern country's research institutes in its field in Poland. Created with the support of the Polish Government, Polish Academy of Sciences (PAN) and UNESCO), the Institute started its activity on January 1, 1999, based on a separate parliamentary bill. Research topics at IIMCB cover the wide area of cancer biology, neurobiology, protein structural biology, intracellular communication, dendritic tree formation, bioinformatics/computer modeling, etc.

Involvement of the International Advisory Board (IAB), the rules of recruitment of all faculty members through international competition and lack of tenure employment make it unique among Polish research institutions. The principles of organization of the Institute are distinct and differ from other research institutes in the country: an important body of the Institute is the International Advisory Board, acting as the Scientific Council; the leader of each research team of the Institute is selected in an international competition; group leader's initial employment is limited to a five-year contract; three years after beginning the research work, progress of research is assessed by the International Advisory Board. There are no permanent scientific positions at the Institute, however a successful lab leader after seven years can be promoted to a senior position and fall into a rolling-tenure mechanism of employment. According to the IIMCB bylaws, the lab leader position constitutes an equivalent of a professorial position of other Polish research institutes.

The Institute is financed in part from the national budget (statutory subvention; budgetary subvention via PAN) and in part from other sources (Ministry of Science and Higher Education), as well as through numerous grants from Foundation for Polish Science, Framework Programs of EU, Max Planck Society, Howard Hughes Medical Institute, European Molecular Biology Organisation, National Institutes of Health, Wellcome Trust, Polish-Norwegian Research Fund, Fogarty International Research Collaboration Center, etc.). More than 80% of funds arrive as competitive grant awards received by the group leaders. IIMCB is involved in various educational programs as well as popularization activities performed by the Centre for Innovative Bioscience Education. To explore commercialization opportunities IIMCB also develops cooperation with some industrial partners with BioTech IP as a special unit (page 5).

Relation of IIMCB to PAN

The International Institute of Molecular and Cell Biology is directly subordinate to the President of the Polish Academy of Sciences (PAN), who supervises the organization and activities of the Institute. The President of PAN nominates members of International Advisory Board (IAB) and the Institute's Directors.

The IIMCB uses a building loaned to it by the Polish Academy of Sciences (PAN). PAN also played a crucial role as a party to the agreement with the Max Planck Society which made it possible to organize international laboratories on both sides.

The Organization of Research at IIMCB

Nine research groups comprised the structure of IIMCB in 2012: Department of Molecular Biology (Żylicz), Laboratory of Bioinformatics and Protein Engineering (Bujnicki), Laboratory of Structural Biology (Bochtler), Laboratory of Neurodegeneration (Kuźnicki), Laboratory of Cell Biology (Miączyńska), Laboratory of Molecular and Cellular Neurobiology (Jaworski), Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden (Paluch), Laboratory of Protein Structure (Nowotny) and Laboratory of Mitochondrial Biogenesis (Chacińska). The research carried out at IIMCB is mainly focused on fundamental biomedical problems. The major research topics include the following:

1. Role of molecular chaperones in cell transformation, including analysis of interactions between human wildtype p53 and mutant p53 with molecular chaperones and oncogenic activity of MDM2 (Żylicz group).
2. Development and application of computer software for structural bioinformatics of proteins and nucleic acids and theoretical and experimental studies of enzymes that act on nucleic acids (protein and RNA structure prediction and modeling, protein engineering, evolutionary analyses, and structure and function determination) (Bujnicki group).
3. Structural and biochemical studies of DNA methylation and hydroxymethylation (Bochtler group).
4. Studies of calcium and β-catenin signaling in the brain and molecular mechanisms of neurodegeneration (Kuźnicki group).
5. Interdependence between endocytic transport, intracellular signal transduction and transcriptional regulation (Miączyńska group).
6. Molecular processes, including gene transcription, kinase dependent cell signaling, cytoskeleton dynamics, intracellular trafficking that underlies neuronal development and plasticity, and central nervous system pathologies (e.g., tuberous sclerosis, epilepsy, and neurodegenerative disorders) (Jaworski group).
7. Mechanics of the actomyosin cortex, study of cortical contractility and the role of cortical mechanics during cytokinesis and migration (Paluch group).
8. Structural and biochemical studies of nucleic acid enzymes (Nowotny group).
9. Biogenesis of mitochondrial proteins, protein transport mechanisms, redox processes in mitochondria (Chacińska group).

Awards and Honors

- **Maciej Żylicz** received the title of **Doctor Honoris Causa of the Jagiellonian University in Kraków** for his significant scientific achievements and outstanding contribution to the development

of biochemistry and molecular biology, especially for his research on the molecular biology of heat shock proteins and their role in cancer transformation, and also for his involvement in the improvement of higher education and the promotion of the good name of Poland and Polish academic centers.

- **Marcin Nowotny** became a **Prime Minister's Award** laureate for the article *Structure of UvrA nucleotide excision repair protein in complex with modified DNA*, published in *Nature Structural & Molecular Biology*, one of the world's most renowned scientific journals.
- **Janusz M. Bujnicki** received an **Award of the Ministry of Science** for outstanding achievements in research.
- **Marcin Nowotny** was awarded an **International Senior Research Fellowship Renewal** from the **Wellcome Trust**. The laureate will develop a project *Structural and biochemical studies of Holliday junction resolution*.
- **Janusz M. Bujnicki** received funding under **the ERC "Proof of Concept" program** to pursue the project *Engineered Sequence-Specific RNases: New Reagents for RNA biotechnology*. The program is a funding scheme which provides funding for researchers who are already ERC grantees, to bridge the gap between research and marketable innovation.
- **Marta Wiśniewska** won the competition for a **group leader position at the Centre of New Technologies, University of Warsaw (CeNT-UW)**; her position will start in June 2013.
- **Urszula Wojda** won an **international competition for the position of Head of Department at the newly established Neurobiology Center at the Nencki Institute**; her position at the Laboratory for Advanced Preclinical Studies will start in April 2013.
- **Marcin Jaciuk** from the Laboratory of Protein Structure received the **Parnas Award** for the best research paper executed in a Polish research facility (in collaboration with **Elżbieta Nowak, Krzysztof Skowronek, Anna Tańska and Marcin Nowotny)** entitled *Structure of UvrA nucleotide excision repair protein in complex with modified DNA,* **published** in the *Nature Structural & Molecular Biology* 2011; 18:191-197.
- **Stanisław Dunin–Horkawicz** from the Laboratory of Bioinformatics and Protein Engineering received a **scholarship for outstanding young scientists** funded by the Ministry of Science and Higher Education.
- **Stanislaw Dunin-Horkawicz**, **Irina Tuszyńska** and **Maria Werner** from the Laboratory of Bioinformatics and Protein Engineering, **Katarzyna Misztal** from the Laboratory of Structural Biology and **Małgorzata Figiel** from the Laboratory of Protein Structure received scholarships for young researchers awarded by the Foundation for Polish Science under the **START Programme**.
- **Ewa Liszewska** from the Laboratory of Molecular and Cellular Neurobiology received a grant from the Foundation for Polish Science under the **Homing Plus Programme** .
- **Anna Urbańska** from the Laboratory of Molecular and Cellular Neurobiology received a **1-year PhD scholarship** awarded under a program implemented by Mazowieckie Province.
- **Małgorzata Figiel** and **Marcin Jaciuk** received funding under the **Iuventus Plus grants (NCN)**.
- **Bartosz Tarkowski** from the Laboratory of Molecular and Cellular Neurobiology received a **Fuga grant (NCN)**.
- **Anna Hupałowska, Anna Malik**, **Małgorzata Perycz** and **Katarzyna Potrzebowska** were **distinguished for their PhD theses** by the Scientific Council of the Nencki Institute.
- **Joanna Gruszczyńska-Biegała** received a **poster award** at the 4th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels held in Isparta, Turkey.

Bio&Technology Innovations Platform

The Bio&Technology Innovations Platform (BioTech-IP) Technology Transfer Office at IIMCB was established in 2010 to support commercialization of inventions and technologies developed by scientists in six public research institutes affiliated to Ochota Biocentre Consortium in Warsaw.

From left: Piotr Potepa, Hubert Ludwiczak, Agnieszka Banrowska, Leszek Lipiński, Adam Sobczak, Magdalena Powierża

BioTech-IP is the first point of contact for companies interested in carrying out a contract research in Ochota Biocentre institutes and for scientists who want to find buyers for their technologies and patents in areas such as biotechnology, biomedicine, bioinformatics, bioengineering, material technologies and bionanotechnology (www.biotech-ip.pl).

Main tasks of the BioTech-IP

- to encourage creative and entrepreneurial attitude in the academic environment by supporting creative activities and promoting commercial exploitation of research results;
- to raise awareness among academics with regard to intellectual property rights through series of lectures and workshops;
- to search for and verify research projects with high commercial potential and commercialize them through spin-off companies formation or licensing of technologies to industrial partners;
- to initiate science-business networking activities and to get in touch with business angels, venture funds and business supporting institutions;
- to promote research services offered by Ochota Biocentre institutes.

2012 events and achievements

ETTbio project launch with European partners

Ten partners (with IIMCB/BioTech-IP among them) from seven European regions launched the ETTBio project (www.ettbio.eu), which aims to identify, exchange and transmit best practices for *Effective Technology Transfer in Biotechnology* in order to improve local and regional policies. Improvement in transfer policies is crucial for creation of new companies, and jobs which leads to higher turnover, as well as increasing regional innovation and economic development.

The project is supported with the funds from the INTERREG IVC programme, which helps regions of Europe share

knowledge and transfer experience to improve regional policies. ETTBio, which has a budget of more than €2 million for the 2012-2014 period, is one of the top priorities for the EU Innovation and Knowledge Economy programme.

PhD scholarships

Nineteen PhD students from six Ochota Biocentre institutes were granted scholarships for their research projects. At a meeting held on October 15, the progress of their work was evaluated by a Scientific Committee which comprised of professors, a patent attorney and an external expert on technology transfer.

Awarded scholarships are funded by the project *Support for bio-tech-med scientists in technology transfer through scholarships, training courses and internships*. The grants are sponsored by Operational Programme - Human Capital co-funded by the European Union under the European Social Fund.

Internship programme

Four scientific researchers were supported with paid internships up to 2 months at R&D companies from the Mazovia region. The internships were sponsored by Operational Programme - Human Capital co-funded by the European Union under the European Social Fund within the project *Support for bio-tech-med scientists in technology transfer through scholarships, training courses and internships*.

Workshops and lectures

BioTech-IP organized a series of lectures and workshops for PhD students and scientists around topics such as soft skills development, patenting and intellectual property rights management, commercialization strategies attended in total by 225 participants.

Conference

On February 28, 2012 a conference on financing research projects was organized. During the conference which promoted the European Social Fund project: *Support for bio-tech-med scientists in technology transfer through scholarships, training courses and internships* the participants got acquainted with open calls for proposals organized by such institutions such as Information Processing Institute, National Centre for Research and Development, National Contact Point for Research Programmes of the European Union and Foundation for Polish Science. The conference also featured the offers from business, namely USP Life Sciences and VENO.

Funds secured for patent protection

BioTech-IP team secured approx. 1 million euro to support PCT patent procedure of 7 technologies originated at Ochota Biocentre within the Operational Programme – Innovative Economy 1.3.2. Support for the protection of industrial property generated in scientific entities as a result of R&D work, co-funded within the European Regional Development Fund. Two of these technologies are owned by IIMCB:

1. *A method of peptide hydrolysis, peptidase, the composition for use as a bacteriostatic and bactericidal agent, a kit and the uses of the active form of LytM from S. aureus or derivatives thereof*
2. *Sequence-specific engineered ribonuclease H and the method for determining the sequence preference of DNA-RNA hybrid binding proteins*

Further 4 new technologies identified in Ochota Biocentre which are subject to international patent procedure were submitted to the last call of proposals within this measure.

Joint science to business projects

BioTech-IP assisted in preparation of six Ochota Biocentre proposals to Applied Research Programme of National Centre for Research and Development. One of these projects is carried out at IIMCB - *Biotechnological applications of bacteriolytic protein* in which a Cooperation Research and Development Agreement was signed with one biotech company for the further improvement of LytM enzyme for commercial purposes.

Management of IP and commercialization of R&D results

BioTech-IP activities resulted in signing a co-ownership agreement between IIMCB and Proteon Pharmaceuticals Ltd. (http://proteonpharma.com), a spin-off company commercializing the invention *Cells and methods useful in characterizing the immunotoxic activity of xenobiotic substances*. This invention is subject to a pending patent procedure in seven European countries and the USA. In 2011 BioTech-IP secured funding to cover the costs of the procedure from the European Regional Development Fund.

The year 2012 was closed with the balance of more than 15 analyses of commercial potential of R&D projects of Ochota Biocentre prepared by BioTech-IP staff. Ten of these projects were subject to feasibility studies and technology offers presented to investors in Poland and abroad. In case of four technologies BioTech-IP was involved in business negotiations with Polish investors, for three other projects the British seed capital fund expressed its preliminary interest.

IT Unit

In accordance with the latest trends in the area of computer network development, the International Institute of Molecular and Cell Biology began the process of virtualization of our servers and network services. This task will aid and optimize the functionality of our hardware as well as allow the implementation of a multitude of operating systems on each physical machine.

From left: Michał Romiszewski, Roman Szczepanowski, Jakub Skaruz

With the support of the Polish Ministry of Science and Higher Education, we have purchased two high performance network servers, which will be used for the task of virtualization of the different services, like E-mail or WWW, and two smaller servers to use as routers and DNS. The disk array and disk array extension, with total available space approx. 30 TB, were the final additions to the system.

New versions of server software like Windows Server 2008R2 and Exchange Server 2010 were purchased, as well as client software for each of 170 computers connected to the network.

We also continued to work on overall development of clusters operated by the Laboratory of Bioinformatics and Protein Engineering: the computing power arrived at 14 TFLOP's (1824 cores), 7.2 TB of RAM memory and an 30 TB of shared file system. 10 Gb Ethernet and 40Gb INFINI *BAND* network communications protocols are used.

Scientific Meetings and Lectures

- The COMBIOM Initial Scientific Meeting, 16.05-17.05.2012 Kiev, Ukraine
- IIMCB Annual Report Session, 15.06.2012, Jachranka, Poland
- Boomerang project "Science for sale?" - meeting with Prof. Barbara Kudrycka, Minister of Science and Higher Education, Warsaw, IIMCB
- Innovative Workshop on Scientific Communication in the framework of the COMBIOM project, 01.10–05.10.2012, Warsaw, IIMCB

Seminars of invited speakers

- **Special Lecture Series: Frontiers of Polish Biosciences***

Wojciech Młynarski (Department of Pediatrics, Oncology, Hematology and Diabetology, Medical University of Lodz, Recipient of the FNP TEAM grant) "Kir6.2/SUR1 ATP potassium channels: from bench to bedside and back to the lab work", 12.04.2012

Paweł Golik (Department of Genetics and Biotechnology, Warsaw University) "PPR proteins and the evolution of nucleo-mitochondrial interactions in yeasts", 14.06.2012

Magda Konarska (Laboratory of Molecular Biology and Biochemistry, The Rockefeller University, New York, USA) "Dynamic RNA-RNA interactions at the spliceosome core and their role in splicing fidelity", 28.06.2012

Bartosz Wilczyński (Institute of Informatics, University of Warsaw) "From ChipSeq data to chromatin interactions: A computer scientist's perspective", 29.11.2012

Bożena Kamińska (Nencki Institute of Experimental Biology, Warsaw) "Molecular pathways in cancer-related inflammation - from molecular mechanisms to novel therapuetic targets", 06.12.2012

- **Lab Leader Competition seminars**

Vladimir P. Korzh (Agency for Science, Technology & Research, Institute of Molecular and Cell Biology, Singapore) "Finding out secrets of the heart… in zebrafish", 19.11.2012

Aniket Gore (National Institutes of Health, Program on Genomics of Differentiation, Bethesda, USA) "Development of blood and vascular system: Lessons from zebrafish", 19.11.2012

- **Regular IIMCB seminars**

Wolfgang Zachariae (Max Planck Institute of Biochemistry, Martinsried, Germany) "How to prevent mitosis in meiosis", 19.01.2012

Maciej Wiznerowicz (Gene Therapy Laboratory, Poznań University of Medical Sciences) "The Cancer Genome Atlas (TCGA) project and induced pluripotent stem cells", 26.01.2012

Jan Riemer (Kaiserslautern University of Technology, Germany) "Oxidative folding and protein homeostasis in mammalian mitochondria", 16.02.2012

Rudolf K. Allemann (School of Chemistry & Cardiff Catalysis Institute, Cardiff University, UK) "From miniature enzymes to biophotonic nanoswitches", 01.03.2012

Mateusz Kolanczyk (Max Planck Institute for Molecular Genetics, Berlin, Germany) "New insights into pathomechanism of NF1 - from bench to bedside", 08.03.2012

Urszula Hibner (IGMM CNRS UMR5535 Montpellier, France) "HCV: viral strategies and cancer", 20.03.2012

Zbyszek Otwinowski (UT Southwestern Medical Center, Dallas, USA) "Quantitative Chip-Seq", 22.03.2012

Polydorides Savvas (Theoretical and Computational Biophysics Group Department of Physics University of Cyprus PO20357, CY1678, Cyprus) "Application of an efficient and accurate generalized Born model to high-throughput computational protein design", 17.04.2012

Agata Klejman (Laboratory of Cell Engineering, Nencki Institute of Experimental Biology, Warsaw) "Genetic modifications in vitro and in vivo. An offer of Nencki Institute Cell Engineering Lab", 19.04.2012

Dirk Sieger (European Molecular Biology Laboratory, Heidelberg, Germany) "From injury to cancer: signaling events guiding leukocytes", 10.05.2012

Abdessamad Zerrouqi (Winship Cancer Institute, Emory University, Atlanta, USA) "New function of the tumor suppressor p14ARF – regulation of tumoral angiogenesis", 17.05.2012

Tomasz Lipniacki (Institute of Fundamental Technological Research PAS, Warsaw) "Mathematical modeling of NF-kappaB system and innate immune responses", 24.05.2012

Anna Sokół (DNA Damage Response Laboratory, Centre for Chromosome Biology, National University of Ireland, Galway) "DNA polymerase eta expression modulates nascent DNA strand length and DDR activation in human cells following platinum-induced DNA damage", 02.07.2012

Hien-Anh Nguyen (Institut de Biologie Structurale, Grenoble, France) "Discovery of a new bacterial protein kinase family: functioning mechanism and cellular roles of YdiB an archetype from Bacillus subtilis", 06.07.2012

Karolina Majorek (Wladek Minor Laboratory, Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, USA) "Structural and functional characterization of selected GNAT superfamily members using semi-high-throughput screening approach", 09.07.2012

Artur Góra (Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the

*A seminar series entitled „Frontiers of Polish Bioscience" was coordinated by Dr. Marta Miączyńska and Dr. Jacek Jaworski. These seminars provided an opportunity to listen to and meet the top Polish scientists who received prestigious awards or grants in a broad field of bioscience.

Environment, Masaryk University, Brno, Czech Republic) "Rational approach for tunnels redesign in enzymes", 11.07.2012

Agnieszka Bondar, Jacek Bernacki and Karol Tymiński (Molecular Diagnostics & Applied Science Roche Diagnostics Polska Sp. z o.o.) "New approach to cell analysis – xCelligence System Roche Diagnostics", 16.07.2012

Vineet Gaur (Department of Biochemistry, The Ohio State University, Columbus, USA) "Stress Associated Proteins: structural and functional insights", 23.07.2012

Ryszard Maleszka (Research School of Biology, The Australian National University, Canberra) "Beyond DNA: how epigenetic mechanisms can generate organismal complexity from a limited number of genes?", 02.08.2012

Elzbieta Glaser (Stockholm University, Sweden) "The mitochondrial peptidasome PreP and Alzheimers disease", 28.08.2012

Alex Bateman (Senior Group Leader, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus Hinxton, Cambridge, UK) "Why biologists should care about Wikipedia" 26.09.2012

Samuel C Flores (Department of Cell and Molecular Biology, Uppsala University, Sweden) "A trajectory of ribosomal translocation with internal coordinate flexible fitting", 26.09.2012

Fyodor Kondrashov (HHMI International Early Career Scientist Center for Genomic Regulation Barcelona, Spain) "My big fat multiple alignment or epistasis as the primary mode of molecular evolution", 01.10.2012

Anna Bartosik (European Molecular Biology Laboratory, Heidelberg, Germany) "Studying protein turnover with fluorescent timers", 04.10.2012

Sabine Rospert (Freiburg University, Germany) "Factors surrounding the exit of the ribosomal polypeptide tunnel guide the early steps of protein biogenesis", 11.10.2012

Tomasz Rygiel (Department of Immunology, Medical University of Warsaw) "Regulation of immune responses by CD200-CD200R axis: a double-edged sword", 18.10.2012

Ewelina Knapska (Nencki Institute of Experimental Biology, Warsaw) "Functional anatomy of neural circuits regulating fear and extinction", 25.10.2012

Agnieszka Kolano (Centre Interdisciplinaire de Recherche en Biologie (CIRB), Centre National de Recherche Scientifique 7241, College de France, Paris) "Interkinetochore tension - Achilles' heel of female meiosis one (MI) in mammals", 08.11.2012

Magdalena Lebiedzińska (Nencki Institute of Experimental Biology, Warsaw) "Mitochondrial dysfunction associated oxidative stress", 13.12.2012

IIMCB researchers' seminars

Michał Boniecki (Laboratory of Bioinformatics and Protein Engineering) "SimRNA - a program for RNA folding simulations", 12.01.2012

Janusz Bujnicki (Laboratory of Bioinformatics and Protein Engineering) "RNA. Or what is that my lab is really doing?", 02.02.2012

Ewa Paluch (Laboratory of Cell Cortex Mechanisms, MPI-CBG Dresden & IIMCB Warsaw) "Actin cortex mechanics and cell shape control in cytokinesis", 09.02.2012

Piotr Brągoszewski (Laboratory of Mitochondrial Biogenesis) "Biogenesis of the mitochondrial intermembrane space proteins - what if something goes wrong?", 23.02.2012

Katarzyna Misztal (Laboratory of Neurodegeneration) "Beta-catenin Wnt-Less journey to the nucleus", 15.03.2012

Elżbieta Nowak (Laboratory of Protein Structure) "Structural studies on the monomeric reverse transcriptase", 26.04.2012

Anna Urbańska (Laboratory of Molecular and Cellular Neurobiology) "Dendrites - lost without ZBP1", 21.05.2012

IIMCB Annual Report Session, 15.06.2012, Jachranka, Poland

Prof. Jarosław Marszałek (The Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk) "Evolutionary biochemistry of mitochondrial Hsp70s" - key note lecture

Agnieszka Górnicka (Laboratory of Mitochondrial Biogenesis) "Ox'n' roll - mitochondrial oxidative folding and the role of ternary complex formation"

Andrzej Nagalski (Laboratory of Neurodegeneration) "Postnatal isoform switch of TCF7L2 and LEF1 transcription factors during the brain development"

Andrew Clark (Laboratory of Cell Cortex Mechanisms) "Thickness and organization of the actin cortex"

Zuzanna Tracz-Gaszewska (Department of Molecular Biology) "Oncogenic function of mutant p53 and molecular chaperones"

Karthik Shanmuganandam (Laboratory of Structural Biology) "Oxidative DNA demethylation in fish?"

Anna Philips (Laboratory of Bioinformatics and Protein Engineering) "New methods for computational prediction of RNA-metal ion and RNA-ligand interactions"

Joanna Gruszczyńska-Biegała (Laboratory of Neurodegeneration) "Complex formation between endogenous STIM2 and ORAI1 in neurons"

Matylda Macias (Laboratory of Molecular and Cellular Neurobiology) "Two faces of rapamycin"

Karolina Górecka (Laboratory of Protein Structure) "Structural studies of Holliday junction resolution"

Agnieszka Mamińska (Laboratory of Cell Biology) "New regulators of cell signaling among endocytic proteins"

Agata Sulej (Laboratory of Bioinformatics and Protein Engineering) "Protein engineering of sequence-specific ribonuclease H and its structural model with the substrate"

Jacek Jaworski (Laboratory of Molecular and Cellular Neurobiology) "CYR61, a matricellular protein is needed for dendritic arborization"

Grants

7th Framework Programme

- **eRNAses** "Engineered Sequence-Specific RNases: New reagents for RNA biotechnology" ERC Proof of Concept; (324624); 149,970 EUR; 2013; **J.M. Bujnicki**
- **BIOMARKAPD** "Biomarkers for Alzheimer's disease and Parkinson's disease"; (2/BIOMARKAPD/JPND/2012); 240,804.27 PLN; 2012-2015; **J. Kuźnicki**
- **BESTCILIA** "Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia"; (305404); 321,720 EUR; 2012-2015; **M. Witt**
- **FishMed** "Fishing for Medicines and their targets using Zebrafish models of human diseases"; (316125); 3,574,100 EUR; 2012-2016; **J. Kuźnicki**
- **TargetSoce** "Pathways of Store-Operated Calcium Entry (SOCE) as a novel therapeutic target in neurodegenerative diseases"; (NCBR/ERA NET RUS/03/2012); 545,623.47 PLN; 2012-2014; **J.Kuźnicki**
- **NERCOMP** "Structural studies of Nucleotide Excision Repair complexes" ERC, (281500); 1,498,000 EUR; 2012-2016; **M. Nowotny**
- **RNA+P=123D** "Breaking the code of RNA sequence-structure-function relationships: New strategies and tools for modelling and engineering of RNA and RNA-protein complexes" ERC, (261351); 1,500,000 EUR; 2011-2015; **J.M. Bujnicki**
- **COMBIOM** "Strengthening Cooperation in Molecular Biomedicine between EU and Ukraine" ERA-WIDE, (294932); 80,036 EUR; 2011-2014; **J. Kuźnicki**
- **EXGENOMES** "Exgenome Molecular Enzymes" Research for SME (286556); 156,000 EUR; 2011-2013; **J.M. Bujnicki**
- **NeuConnect** "Novel strategies for the treatment of schizophrenia based on genetic variation of the neural cell adhesion molecule NCAM and enzymes involved in its posttranslational modifications" (ERA-NET-NEURON/01/2011); 973,080 PLN; 2011-2014; **J. Kuźnicki/M. Wiśniewska**
- **AMPREPACELL** "Development of new experimental models for mental retardation and autism by iPS technology: generation of human affected and animal model neurons by reprogramming skin fibroblasts and testing gene correction using in vitro and in vivo models" (ERA-NET-NEURON/03/2011); 1,419,075 PLN; 2011-2014; **J. Jaworski**
- **ImageNinND** "Imaging Neurogenesis in Neurodegenerative Disease: In vivo imaging of dopaminergic adult-born neurons in the olfactory bulb of animal models of Parkinson's disease" (ERA-NET-NEURON/03/2010); 1,085,875 PLN; 2010-2013; **J. Jaworski**
- **TRANSPOL** "Transport and signalling mechanism in polarized cells" ITN, (264399); 225,523 EUR; matching funds 475,200 PLN; 2010-2014; **M. Miączyńska**
- **HEALTH-PROT** "Proteins in Health and Disease" Research Potential, (229676); 954,100 EUR; matching funds 4,099,289 PLN; 2009-2012; **J. Kuźnicki**
- **SBMPs** "Structural Biology of Membrane Proteins" ITN, (211800); 263,284 EUR; matching funds 870,120 PLN; 2008-2012; **S. Filipek**

Other International Funds

- Wellcome Trust International Senior Research Fellowship "Structural and Biochemical studies of Holliday junction resolution" (0988022); 3,369,854 PLN; 2013-2018; **M. Nowotny**
- Howard Hughes Medical Institute, International Early Career Award "Structural and Mechanistic Studies of Nucleic Acid Processing"; 715,000 USD; 2012-2017; **M. Nowotny**
- Polish Swiss Research Fund "The role of tumor susceptibility gene 101 (Tsg101) in transcriptional regulation in health and disease" (PSPB-094/2010); 5,365,200 PLN; 2012-2016; **M. Miączyńska**
- International Centre for Genetic Engineering and Biotechnology, "mTOR-driven phosphorylation of ZBP1 and Ago2 in neuronal development" (CRP/12/010); 48,000 EUR; 2012-2015; **J. Jaworski**
- INTERREG IV C, ETTBio "Effective Technology Transfer in Biotechnology"; (1210R4); 128,070 EUR; 2012-2014; **M. Powierża**
- DFG Program Sensory and Regulatory RNAs in Prokaryotes "Single-molecule fluorescence analysis of the temperature dependent structure and dynamics of an RNA thermometer: consequences for its molecular function" (SE 1195/12-2); 90,450 EUR; 2010-2013; **J.M. Bujnicki**
- EMBO Installation Grant "Protein biogenesis and redox homeostasis in mitochondria" (1966); 250,000 EUR; 2010-2014, **A. Chacińska**
- EMBO Installation Grant "Structural and biochemical studies of UvrA DNA repair protein" (1476); 250,000 EUR; 2007-2012; **M. Nowotny**
- Wellcome Trust International Senior Research Fellowship "Structural and functional studies of two members of integrase superfamily – type 2 RNase H and Ruvc resolvase – from substrate recognition to catalysis" (081760); 4,106,806 PLN; 2007-2013; **M. Nowotny**
- Wellcome Trust International Senior Research Fellowship "Communication between intracellular organelles in trafficking and signalling: The role of APPL proteins" (076469); 4,315,706 PLN; 2005-2012; **M. Miączyńska**

Structural Funds

- IE OP 1.2. FNP Programme **HOMING PLUS** "Modeling tuberous sclerosis with induced pluripotent steam cells"; (HOMING PLUS/2012-5/6); 302,000 PLN; 2013-2014; **E. Liszewska**
- IE OP 1.2. FNP Programme **POMOST** "Characterization of selected endocytic proteins as novel regulators of AP-1 mediated transcription" (POMOST/2011-3/11); 430,000 PLN; 2012-2014; **E. Szymańska**
- IE OP 1.1.2 FNP **TEAM** Programme "Structural biology of methylation and hydroxymethylation"; (TEAM/2010-6/1); 2,023,940 PLN; 2011-2015; **M. Bochtler**
- IE OP 1.2 FNP Programme **VENTURES** "The acquisition of chemotherapy resistance in non-small cell lung cancer – role of the p53 family proteins" (VENTURES/2010-6/8) 231,000 PLN; 2011-2014; **Z. Tracz**
- IE OP 1.1.2 FNP **TEAM** Programme "Modeling of RNA and protein-RNA complexes: from sequence to structure to function"; (TEAM/2009-4/2); 2,200,000 PLN; 2010-2014; **J.M. Bujnicki**
- IE OP 1.1.2 FNP Programme **MPD** "PhD Programme in Molecular Biology: Studies of nucleic acids and proteins – from basic to applied research"; (MPD/2009-3/2); 2,265,421 PLN; 2010-2015; **M. Witt** (7 PhD fellowships for all group leaders, see page 87)
- HC OP 8.2.1 MJWPU "Support for bio tech med. scientists in technology transfer" (UDA-POKL.08.02.01-14-041/09-00); 2,586,221 PLN; 2010-2013; **M. Powierża**
- IE OP 1.2. FNP Programme **POMOST** „Functional characterization of the interactions between endosomal adaptor proteins APPL and Dvl proteins in the Wnt signaling pathway"(POMOST/2010-1/1); 420,000 PLN; 2010-2013; **M. Banach-Orłowska**
- IE OP 1.1.2 FNP Programme **WELCOME** "Biogenesis and turnover of mitochondria intermembrane space proteins" (WELCOME/2009/1); 5,940,670 PLN; 2009-2014; **A. Chacińska**
- IE OP 2.2.3 NCBR "Biocentrum Ochota – IT infrastructure for development of strategic directions of the biology and medicine", (POIG.02.03.00-00-003/09); 4,834,300 PLN; 2009-2013; **J.M. Bujnicki** and **S. Filipek**
- IE OP 2.2.2 NCBR "Centre of Pre-clinical Research and Technology (CePT)" (POIG.02.02.00-14-024/08-00); 14,625,545 PLN; 2008–2013; **J. Kuźnicki**
- IE OP 1.3.2 OPI "Support for patent procedure of the invention: Tools and methods useful in characterising the immunotoxic activity of xenobiotic substances" (UDA-POIG.01.03.02-00-063/10-00); 230,315 PLN; 2011-2015; **M. Powierża**

NCBiR Research Grants

- "Biotechnological applications of bacteriolytic protein"; (177126); 2,059,000 PLN (total grant budget: 2,443,260 PLN); 2013-2015; Coordinator **I. Sabała**
- "New drugs for targeted therapy of multiple myelomas"; (176911); 368,880 PLN (total grant budget: 5,327,452 PLN); 2012-2015; **M. Nowotny** (partner); Coordinator: A. Dziembowski, IBB PAN
- "Polish reference genome for genomic diagnostics and personalized medicine"; (181852); 732,347 PLN (total grant budget: 4,648,937 PLN); 2013-2016; **M. Mossakowska** (partner); Coordinator: Genomed S.A.
- AriaDNA 2010 Project (OR00002712); 2,613,152 PLN (total grant budget: 9,904,670 PLN); 2010-2013; **J. Kuźnicki**

NCN Research Grants

- **MAESTRO** "Molecular mechanisms of pro-survival processes in breast cancer"; 3,000,000 PLN; 2013-2017; **M. Żylicz**
- **OPUS** "Interplay between MIA pathway and reactive oxygen species in mitochondrial homeostasis"; (2012/05/B/NZ3/00781); 663,500 PLN; 2013-2016; **M. Wasilewski**
- **OPUS**"Nuclear functions of mTOR in neurons"; (2012/05/B/NZ3/00429); 750,000 PLN; 2013-2015; **J. Jaworski**
- **PRELUDIUM** "Genome wide high throughput analysis of 5-hydroxymethyl cytosine in Danio rerio"; (2012/05/N/NZ2/02233); 150,000 PLN; 2013-2016; **K. Shanmuganandam**
- **PRELUDIUM** "Structural basis of the recognition of postreplicative DNA modifications"; (2012/05/N/NZ1/01912); 100,000 PLN; 2013-2015; **W. Siwek**
- **PRELUDIUM** "Analysis role of the PsbS subunit from photosystem II in the non-photochemical quenching"; (2012/05/N/NZ1/01922); 99,200 PLN; 2013-2015; **P. Haniewicz**
- **PRELUDIUM** "Modeling of charge transport in RNA structural motifs"; (2012/05/N/NZ1/02970); 75,000 PLN; 2013-2014; **J. Stasiewicz**
- **SONATA** "Identification of post-transcriptional modifications in RNA sequences through mass spectrometry"; (2012/05/D/ST/6/0382); 493,125 PLN; 2013-2016; **B. Kluge**
- **MAESTRO** "Structural RNomics"; (2012/04/A/NZ2/00455); 3,000,000 PLN; 2012-2017; **J.M. Bujnicki**
- **PRELUDIUM** "Development of a new scoring function for models of protein-small molecule complexes and its use for studying the mechanism of protein-ligand recognition"; (2011/03/N/NZ2/03241); 230,000 PLN, 2012-2014, **I. Tuszyńska**
- **OPUS** "Oxidation landscape of mitochondrial proteins upon ROS production and in ageing"; (2011/02/B/NZ2/01402); 997,500 PLN; 2012-2015; **A. Chacińska**
- **PRELUDIUM** "The interplay between the processes of inner membrane formation and protein transport in mitochondria"; (2011/03/N/NZ3/01614); 318,750 PLN; 2012-2015 **P. Kwiatkowska**
- **OPUS** "The role of Amyloid Precursor Protein in the regulation of Store-Operated Calcium Entry"; (2011/03/B/NZ3/01760); 504,000 PLN; 2012-2015; **T. Węgierski**

- **OPUS** "Regulation of clathrin-dependent endocytosis by mTOR kinase in neuronal development"; (2011/03/B/NZ3/01970); 813,125 PLN; 2012-2015; **J. Jaworski**
- **SONATA** "Determination of composition structure and substrate specificity of the mRNA_m6A methyltransferase protein complex"; (2011/03/D/NZ1/03247); 750,000 PLN; 2012-2015; **E. Purta**
- **OPUS** "The canonical Wnt signaling pathway in the development of the thalamus"; (2011/03/B/NZ3/04480); 842,500 PLN; 2012-2015; **M. Wiśniewska**
- **FUGA** "Does the hyperactivation of mTOR kinase interfere with cell differentiation into neurons?"; (2012/04/S/NZ3/00264); 608,100 PLN; 2012-2015; **B. Tarkowski**
- **FUGA** "A code for RNA recognition in RNA–RRM interactions"; (2012/04/S/NZ1/00729); 612,000 PLN; 2012-2015; **M. Nowacka**
- **SONATA** "Architecture and evolution of protein-RNA networks and their relevance in the process of virulence regulation"; (2011/03/D/NZ8/03011); 720,000 PLN; 2012-2016; **S. Dunin-Horkawicz**
- **MAESTRO** "Transgenic mice with elevated basal level of calcium ions in neurons as a model of aged-induced neurodegeneration of sporadic Alzheimer's disease"; (2011/02/A/NZ3/00144); 2,989,800 PLN; 2012- 2017; **J. Kuźnicki**
- **MAESTRO** "New functions of endocytic proteins in transcriptional regulation"; 2,875,000 PLN; 2012-2017; **M. Miączyńska**
- **HARMONIA** "The relationship between GSK3α and GSK3β activities and neuronal plasticity in chronic stress" (2011/01/M/NZ3/05413); 499,964 PLN; 2011-2014; **I. Cymerman**
- **PRELUDIUM** "Defining the mechanism of GSK3 dependent regulation of mTOR kinase activity in neurons in physiology and pathology" (2011/01/N/NZ3/05409); 150,000 PLN; 2011-2014; **M. Urbańska**
- **PRELUDIUM** "Characterization of mTOR-dependent phosphorylation of ZBP1 and CLIP-170 and description of its contribution to dendritic arbor development" (2011/01/N/NZ3/05405); 180,000 PLN; 2011-2014; **A. Urbańska**
- **OPUS** "Investigation of arc protein role in synaptic plasticity of hippocampal and cortical neurons and mechanisms regulating arc expression with the special focus on GSK3-dependent degradation" (2011/01/B/NZ3/05397); 450,000 PLN; 2011-2014; **A. Goździ**
- **PRELUDIUM** "Role of transcription factor TCF7L2 in establishment of thalamocortical connectivity and identity of thalamic neurons" (2011/01/N/NZ3/05345); 96,000 PLN; 2011-2013; **A. Nagalski**
- **SONATA** "The contribution of STIM proteins and the role of Store Operated Calcium Entry (SOCE) in calcium homeostasis of neurons" (2011/01/D/NZ3/02051); 684,000 PLN; 2011-2016; **J. Gruszczyńska-Biegała**
- **SONATA** "Structural and functional characterization of novel non-coding RNAs from Helicobacter pylori" (2011/01/D/NZ1/00212); 550,000 PLN; 2011-2014; **G. Chojnowski**
- **PRELUDIUM** "Generation of knockouts of HMTR1 and HMTR2 genes in human somatic cells and functional analysis of cap1 and cap2 methyltransferases encoded by these genes" (2011/01/N/NZ1/00211); 100,000 PLN; 2011-2013; **M. Werner**
- **OPUS** "Sequence specificity and its determinants in dsRNA endoribonucleases" (2011/01/B/NZ1/00209); 350,000 PLN; 2011-2014; **K. Skowronek**
- **PRELUDIUM** "The role of HSP70 in the stabilization of p53 mutants in cancer cells" (2011/01/N/NZ1/00202); 192,000 PLN; 2011-2013; **M. Wiech**

Ministerial Research Grants

- "Structural analysis of RNase H3 in complex with a substrate - the mechanism of action and substrate specificity in the context of an enzyme family" (IP2011060971); 150,000 PLN; 2012-2013; **M. Figiel**
- "Development and application of new methods for protein-RNA and protein-DNA complexes modeling" (IP2011057071); 175,000 PLN; 2012-2014; **I. Tuszyńska**
- "Structural studies of mechanism of action of UvrC protein from bacterial DNA repair system called nucleotide excision repair system" (IP2011018671); 150,000 PLN; 2012-2013, **M. Jaciuk**
- "Structural analysis of the RNA-RNA and RNA-protein interactions" (IP2011006671); 145,000 PLN; 2012-2013; **G. Chojnowski**
- "Practical algorithms for graph isomorphism testing in the computational biology" (IP2011058671); 160,000 PLN; 2012-2013; **T. Waleń**
- "Casimir-Polder effect in scattering of atoms on liquid surfaces" (IP2011030771); 150,000 PLN; 2012-2013; **G. Łach**
- "Coordinating proteasome subunit expression: structural biology of the master regulator Rpn4" (IP2011050971); 400,000 PLN; 2012-2013; **M. Sokołowska**
- "Structural biology of anti-cancer DNA methyltransferase inhibitors" (IP2011060971); 200,000 PLN; 2012-2013; **M. Wojciechowski**
- "Bioinformatics analysis of sequence-structure-function relationships in the GIY-YIG nuclease superfamily" (IP2011021871); 100,000 PLN; 2012-2012; **K. Kamińska**
- "Analysis of the relationship between sequence and structure in coiled-coil protein domains" (IP2011011071); 178,000 PLN; 2012-2014; **S. Dunin-Horkawicz**
- "Changes in cell cycle and apoptosis as a basis for diagnosis and potential therapeutic targets in Alzheimer's disease" (NN401596840); 408,000 PLN; 2011-2014; **U. Wojda**
- "Is there a "universal" RNA-guided DNA endonuclease?" (NN302654640); 400,000 PLN; 2011-2014; **M. Bochtler**
- „The Elmo1 function of regulated by mTOR kinase activity in neuronal plasticity" (NN301779040); 250,000 PLN; 2011-2014; **M. Błażejczyk**
- "Function of STIM proteins in capacitative calcium entry to the ER of healthy neurons and of cells with calcium dyshomeostasis in Alzheimer's disease" (NN301190039); 480,000 PLN; 2010-2013; **J. Kuźnicki**
- „The role of multifunctional adaptor proteins APPL1 and APPL2 in the regulation of cell growth and tumorigenic potential" (NN301189839); 336,000 PLN; 2010-2013; **B. Pyrzyńska**
- "Experimental characterization of hMTcap1 and hMTcap2 – last missing enzymes taking part in biosynthesis of the cap

structure of human mRNA" (NN301425338); 500,000 PLN; 2010-2013; **J.M. Bujnicki**

- "Biochemical and structural studies of lentiviral reverse transcriptases" (NN301439738); 599,800 PLN; 2010-2014; **M. Nowotny**
- "Mechanism of oncogenic activities of mutated TP53" (NN302621838); 600,000 PLN; 2010-2013; **A. Żylicz**
- "Identifiction of the genetic program activated by Lef1/β-catenin complex in mature neurons" (NN301424538); 372,000 PLN; 2010-2013; **M. Wiśniewska**
- "Structural studies of ββα-Me restriction endonucleases" (NN3014250378); 400,000 PLN; 2010-2012; **H. Czapińska**
- "Towards a new drug against influenza: Identification and characterization of compounds which abolish the activity of the influenza virus mRNA polymerase by the inhibition of virus endonuclease" (NN401585738); 150,000 PLN; 2010-2013; **K.H. Kamińska**
- "Innovation Creator (Kreator Innowacyjności) – to encourage entrepreneurship among scientists"(31/PMKI/U/30-06.09/2010); 422,990 PLN; 2010-2013; **M. Powierża**
- "The role of mitochondria in biogenesis and pathogenesis of superoxide dismutase Sod1" (NN301298337); 476,000 PLN; 2009-2012; **A. Chacińska**
- "Identification and characteristics of endocytic proteins involved in regulation of gene transcription" (NN301296437); 340,740 PLN; 2009-2013; **I. Pilecka / M. Miączyńska**
- International Project Grant (MPG Program) "The role of cell cortex mechanics in cell motility" (454/N-MPG/2009/0); 4,692,929 PLN; 2009-2012; **E. Paluch**

Ministerial Doctoral Grants

- "Searching for β-catenin regulators in thalamic neurons" (NN301191739); 48,000 PLN; 2010-2012; **J. Kuźnicki/K. Misztal**
- "Function of calmyrin 2 in Ca2+-dependent exocytosis" (NN301335239); 60,000 PLN; 2010-2012; **U. Wojda/K. Dębowska**
- "Functional analysis of proteins responsible for processing of mRNA 3'termini. Identification of domains and intrinsically disordered regions" (NN301190139); 37,600 PLN; 2010-2012; **J.M. Bujnicki/L. Kozłowski**

Ministerial Commissioned Grants

- PolSenior "Ageing of the Polish population – medical, psychological, sociological and economic aspects" (PBZMEiN- 9/2/2006); 12,178,420 PLN; 2007-2012; Director: P. Błędowski (SGH, Warsaw School of Economics), coordinator **M. Mossakowska**

Doctoral Degrees in 2012

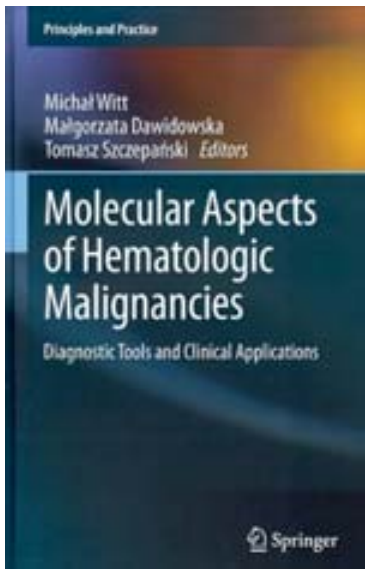
- **Emilia Białopiotrowicz**, PhD thesis: „Function of calmyrin 1 and calmyrin 2 in neuronal calcium signaling". Thesis advisor: U. Wojda; 14.03.2012, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- **Marek Wojciechowski**, PhD thesis: „Biochemical and structural characterization of R. Thal and M. Mpel". Thesis advisor: M. Bochtler; 22.05.2012, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- **Maciej Geremek**, PhD thesis: „The classical genetic and genomic approach to the pathogenesis of primary ciliary dyskinesia". Thesis advisors: M. Witt, C. Wijmenga, 13.06.2012, University of Groningen
- **Anna Hupałowska**, PhD thesis: „The role of APPL1 protein in the regulation of the NF-κB pathway". Thesis advisor: M. Miączyńska; 21.06.2012, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- **Anna Malik**, PhD thesis: „RNA libraries as a tool to search novel mTOR-regulated proteins important for neuronal physiology and pathology". Thesis advisor: J. Jaworski, 26.06.2012, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- **Katarzyna Potrzebowska (Dębowska)**, PhD thesis: „Search for diagnostic markers in Alzheimer's disease based on the analysis of the cell cycle and apoptosis in human lymphocytes B". Thesis advisor: U. Wojda, 11.09.2012, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
- **Wojciech Potrzebowski**, PhD thesis: „Macromolecular complexes modeling using low-resolution experimental data". Thesis advisor: J.M. Bujnicki, 23.10.2012, Institute of Biochemistry and Biophysics, Warsaw, Poland
- **Małgorzata Perycz**, PhD thesis: „Role of ZBP-1 in dendritogenesis of hippocampal neurons". Thesis advisor: J. Jaworski, 15.11.2012, Nencki Institute of Experimental Biology PAN, Warsaw, Poland

Publications developed by staff outside research lab teams

- Laczmanski L, Milewicz A, Lwow F, Puzianowska-Kuznicka M, Pawlak M, Kolackov K, Jedrzejuk D, Krzyzanowska-Swiniarska B, Bar-Andziak E, Chudek J, **Mossakowska M**. Vitamin D receptor gene polymorphism and cardiovascular risk variables in elderly Polish subjects. Gynecol Endocrinol, 2012 [Epub ahead of print]
- **Bukowy-Bieryłło Z**, Ziętkiewicz E, Loges NT, Wittmer M, Geremek M, Olbrich H, Fliegauf M, Voelkel K, Rutkiewicz E, Rutland J, Morgan L, Pogorzelski A, Martin J, Haan E, Berger W, Omran H, **Witt M**. RPGR mutations might cause reduced orientation of respiratory cilia. Pediatr Pulmonol, 2012 [Epub ahead of print]
- Skalska A, Wizner B, Piotrowicz K, Klich-Rączka A, Klimek E, **Mossakowska M**, Rowiński R, Kozak-Szkopek E, Jóźwiak A, Gąsowski J, Grodzicki T. The prevalence of falls and their relation to visual and hearing impairments among a nation-wide cohort of older Poles. Exp Gerontol, 2012 [Epub ahead of print]
- Ziętkiewicz E, **Bukowy-Bieryłło Z**, Voelkel K, Klimek B, Dmeńska H, Pogorzelski A, Sulikowska-Rowińska A, Rutkiewicz E, **Witt M**. Mutations in radial spoke head genes and ultrastructural cilia defects in East-European cohort of primary ciliary dyskinesia patients. PLoS One, 2012; 7(3):e33667
- Kraszewska MD, Dawidowska M, Kosmalska M, Sędek L, Grzeszczak W, Szczepański T, **Witt M**. DNA methylation pattern is altered in childhood T-cell acute lymphoblastic leukemia patients as compared with normal thymic subsets: insights into CpG island methylator phenotype in T-ALL. Leukemia, 2012; 26:367–371
- Kraszewska MD, Dawidowska M, Szczepanski T, **Witt M**. T-cell acute lymphoblastic leukaemia: recent molecular biology findings. Br J Haematol, 2012;156(3):303-315
- Kraszewska MD, Dawidowska M, Kosmalska M, Sędek L, Grzeszczak W, Szczepański T, **Witt M**. Immunoglobulin/T-cell receptor gene rearrangements in the diagnostic paradigm of pediatric T-cell acute lymphoblastic leukemia patients. Leukemia & Lymphoma, 2012; 53(7):1425-8
- Ziętkiewicz E, Witt M, Daca P, Zebracka-Gala J, Goniewicz M, Jarząb B, **Witt M**. Current genetic methodologies in the identification of disaster victims and in forensic analysis. J Appl Genet, 2012; 53(1):41-60.

„Molecular Aspects of Hematologic Malignancies"

Molecular Aspects of Hematologic Malignancies. Diagnostic tools and clinical applications, is a book edited by Michał Witt, Małgorzata Dawidowska and Tomasz Szczepański, published within a series *Principles and Practice* in June 2012 by Springer Verlag, Heidelberg. This monography provides a state-of-the-art approach to the molecular basis of hematologic diseases encompassing a set of representative hematologic diseases of children and adults: acute lymphoblastic leukemia, acute myeloid leukemia, B-cell Non-Hodgkin lymphomas, multiple myeloma, chronic lymphocytic leukemia, myelodysplastic syndromes and myeloproliferative neoplasms. Significant part of the book is devoted to contemporary procedures of bone marrow transplantation



and molecular monitoring of patients treated with this method: donor-recipient matching, banking of biological material, analyses of post-transplant chimerism, and minimal residual disease monitoring. The volume concludes with a practical section comprising step-by-step protocols of molecular techniques in hematology. The authors of 29 individual chapters (476 pages altogether) are well recognized experts in the field, from Poland and other countries. This volume is a significantly extended and totally newly written continuation of the concept of the previous book of the same editors ("Hematologia molekularna"), conceived within a grant coordinated at our Institute and published in 2009 in Polish under the auspices of IIMCB.

Details of Selected Projects

Selected Projects

7th Framework Programme grants

IIMCB scientists are laureates of four ERC grants - three ERC Starting Grants (StG) and the Proof of Concept grant. Two of the ERC StG, of **Prof. Bujnicki** and **Dr. Nowotny**, are among ten such projects awarded to Polish scientists and implemented in Poland until 2012. The third one will be implemented at the University College London by **Dr. Ewa Paluch**. IIMCB, as the only Polish institute, received support to develop ERC Proof of Concept grant. The unique value that each of these projects represents is further enhanced by complementarities and synergies between the three of them. This can considerably increase the impact of the implemented projects and advance science towards even more exciting discoveries.

RNA+P=123D, ERC Starting Grant, FP7

The project „Breaking the code of RNA sequence-structure-function relationships: New strategies and tools for modelling and engineering of DNA and RNA-protein complexes" was awarded to **Prof. Janusz M. Bujnicki**, the first laureate of this prestigious EU grant at IIMCB. The aim of the 5-year project is to use bioinformatics and experimental methods to crack the code of sequence-structure relationships in RNA and RNA-protein complexes and to revolutionize the field of RNA & RNP modelling and structure/function analyses.

eRNAses, ERC Proof of Concept, FP7

Awarded to **Prof. Janusz M. Bujnicki**, the eRNAses project complements the objectives set out in the RNA+P=123D ERC StG. The ERC Proof of Concept Grant is designed for 12 months. It aims at rationally constructing RNA restriction enzymes with defined sequence specificity. Establishing such a novel technology platform would not only revolutionize both the basic and applied science of RNA but also heavily influence other disciplines utilizing modern molecular biology tools. To exploit the full potential of this technology, the idea is to start the commercialization process immediately. These two projects are closely synergistic, both in terms of their research objectives and the tools used.

NERCOMP, ERC Starting Grant, FP7

The laureate of the ERC StG project "Structural studies of Nucleotide Excision Repair complexes" is **Dr. Marcin Nowotny**. The overall objective of NERCOMP is to expand knowledge about the DNA repair mechanisms. Specifically, Dr. Nowotny focuses on the structural and biochemical characterization of protein complexes involved in the NER pathways in bacteria and eukaryotes. This is a key process not only in cognitive terms, but also because a disturbance in these mechanisms in humans can entail tumorigenesis.

MORPHOCORDIV, ERC Starting Grant, FP7

Dr. Ewa Paluch became the ERC laureate to implement project „The inherent morphological potential of the actin cortex and the mechanics of shape control during cell division". The main focus of Dr. Paluch group is to understand how the mechanical properties of a cell are regulated to give a certain shape to a cell.

BESTCILIA, Cooperation project, FP7 Health

IIMCB is one of eight Polish institutes which received funding under the FP7 Health call in 2012. IIMCB's awardee is **Prof. Michał Witt** who participates in a three-year small-scale research project "Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia". Coordinated by Prof. Heymut Omran from the University of Munster, this multi-partner project concentrates on observational trials to characterize the clinical course and improve the diagnosis and treatment of Primary Ciliary Dyskinesia (PCD). Prof. Witt's responsibilities in BESTICILIA are to supervise observational trials carried out by a third party, the Institute of Tuberculosis and Lung Diseases in Rabka-Zdrój, as well as to lead Project's training and dissemination activities.

BIOMARKAPD, EU Joint Programming in Neurodegenerative Diseases, FP7

IIMCB got involved in the Joint Programming in Neurodegenerative Diseases (JPND), the first EU initiative of this kind. Prof. Jacek Kuźnicki and his team participate in a three-year, multi-partner project "Biomarkers for Alzheimer's disease and Parkinson's disease". The idea of BIOMARKAPD is to standardise the assessment of established and new fluid biomarkers for AD and PD. Prof. Kuźnicki's tasks focus on testing new AD and PD markers, as well as on coordinating the research activities of the four Polish project teams led, respectively, by: Prof. Szczudlik from Jagiellonian University's College of Medicine, Prof. Gabryelewicz from Mossakowski Medical Research Centre, Prof. Zboch from Wrocław Medical University and Prof. Barbara Mroczko from Medical University of Białystok.

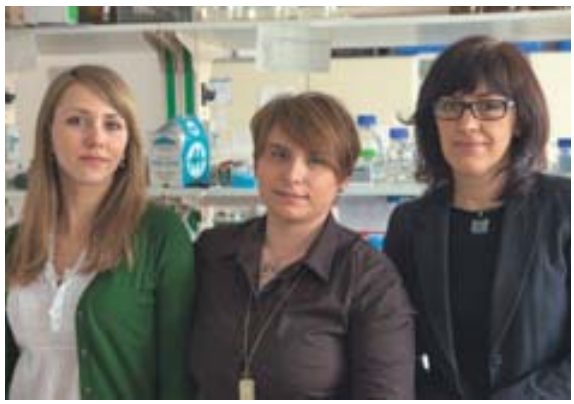
TargetSOCE, Era-Net-Rus, FP7

In response to the FP7 Era-Net-Rus pilot call aiming at intensifying and strengthening S&T cooperation between Russia and Europe, **Prof. Jacek Kuźnicki** initiated a coordination of a three-partner project consortium "Pathways of Store-Operated Calcium Entry (SOCE) as a novel therapeutic target in neurodegenerative diseases". Within two years, together with Prof. Methner from the University of Mainz and Prof. Kaznacheyeva from the Institute of Cytology, Russian Academy of Sciences, new target proteins of pathways involved in calcium homeostasis will be studied as a potential site for new treatments – both diseases are accompanied by profound changes in the intracellular Ca^{2+} homeostasis.

Projects outside research lab teams

As a complement to the basic activities of regular IIMCB laboratories, two smaller independent research teams operate at the Institute, running their own research projects. The creation of these groups, in parallel to the normal recruitment policy of IIMCB, arose from the need to use the existing expertise and highly qualified researchers and to enable the development of topics relevant to the Institute and of particular importance for domestic science. Each of these groups carries out highly-funded research projects, which are an important part of IIMCB's research policy.

A group headed by **Dr. Izabela Sabała** carries out a 3-year **project *Biotechnological applications of bacteriolytic protein (Aurezyna)*** which is financed by the National Center for Research and Development **under the Applied Research Program.** The funding was awarded to a consortium established by IIMCB (project leader) and A&A Biotechnology (commercial partner). While working on structural and biochemical characterization of an autolysin from *Staphylococcus aureus*, very unusual and commercially valuable features of the enzyme were discovered: very efficient lysis of staphylococcal cells in unique environmental conditions of low temperature and exceptionally low ionic strength. The aim of the project is to explore commercial applications of the enzyme ranging from staphylococcal cell lysis allowing isolation of cellular components, through diagnostic tests, to a wide range of bacteriostatic and bacteriolytic applications, e.g. to eliminate staphylococci from food and hospital environment. Further basic research will be also carried out to expand environmental tolerance of the enzyme and modify its specificity.



For the past 14 years, the social, psychological, economic and clinical aspects of rapid ageing of today's societies – a process resulting mainly from an increasing life span and declining birth rates has been studied at IIMCB. The *PolStu* lead by Prof. Jacek Kuźnicki (on Polish centenarians) and multicenter *PolSenior* coordinated by **Dr. Małgorzata Mossakowska** (on Polish elderly people) *Projects* became a great success. The results of both projects facilitated prioritizing the state's public health and social policies aimed at the elderly population. Currently, the group is involved in a 3-year project named ***Polish Reference Genome for Genomic Diagnostics and Personalized Medicine (PlGen)***, financed by NCBiR. The project aims at determination of the reference sequence and completing of genomic databases of Polish subpopulations for commercial diagnostic applications and research in the field of personalized medicine. The project will be carried out with the use of, among others, the biological material and clinical data yielded by the *PolStu* and *PolSenior* projects, and a genetic basis of successful aging will be of particular interest to researchers.

Cooperation with Other Institutions

Domestic Cooperation

Biocentrum Ochota (www.biocentrumochota.gov.pl)

In January 2008, the scientific activities of the Biocentrum Ochota Consortium of the Polish Academy of Sciences were launched at the initiative of six research institutes operating at the Ochota Campus in Warsaw.

The founders and members of the Consortium are:

1. International Institute of Molecular and Cell Biology
2. Institute of Biochemistry and Biophysics PAN
3. Nałęcz Institute of Biocybernetics and Biomedical Engineering PAN
4. Nencki Institute of Experimental Biology PAN
5. Mossakowski Medical Research Centre PAN
6. Institute of Fundamental Technological Research PAN

The basic principle behind Biocentrum Ochota is that the considerable scientific potential, represented by the combined group of experts working in these six institutes, should be pooled and used for the development of large-scale research projects that go beyond the capabilities of individual units. The implementation of such projects will overlap with the statutory research areas of individual institutes in the fields of biology, medicine and bioengineering. Pooling the resources and expertise of individual institutions will also aid the acquisition of financial backing, including European Union grants under the Operational Programme – Innovative Economy and the Operational Programme – Human Capital, co-financed by the European Social Fund.

The EU funds obtained by Biocentrum Ochota are used not only for research projects but also to expand the team of researchers. The scientists from the member institutions of Biocentrum Ochota are specialists recognized on the national and international arena as experts in their fields. This is evidenced by a broad spectrum of scientific cooperation with Polish and foreign research centres, by numerous invitations to participate in projects, symposia, conferences and publications, and by the volume of scientific output. Researchers at Biocentrum Ochota have also received many awards at home and abroad, including the most prestigious awards for scientific achievements, awarded annually by the Foundation for Polish Science.

University of Gdańsk

In October 2011, an agreement was signed between the Intercollegiate Faculty of Biotechnology at the University of Gdańsk/Medical University of Gdańsk and the IIMCB, regarding IIMCB's accession to Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS) at the University of Gdańsk. LiSMIDoS was originally an initiative of the councils of four faculties at the University of Gdańsk, namely: the Intercollegiate Faculty of Biotechnology UG/MUG, the Faculty of Biology, the Faculty of Chemistry and the Faculty of Mathematics, Physics and Informatics. The major objective is to provide to PhD students a programme of interdisciplinary training that will allow them to work in today's competitive scientific environment which very often requires cross-disciplinary expertise. The studies will prepare candidates wishing to obtain a PhD degree in the area of biological sciences (biology and biochemistry), chemical sciences (chemistry), physical sciences (physics) and mathematical sciences (mathematics). First IIMCB students will start their education in 2013. Prof. Janusz M. Bujnicki and Dr. Jacek Jaworski have been appointed members of LiSMIDoS Programme Council. Further developments are being planned for future applications together with the Intercollegiate Faculty of Biotechnology at the University of Gdańsk.

Museum and Institute of Zoology PAN (MIZ)

An agreement was reached to set up a joint research group between MIZ and IIMCB, centred on the sequencing capabilities (PacBio) acquired by MIZ where the laboratory will be located. The greatest overlap in scientific interest of both institutes is seen in the field of modern sequencing, and members of IIMCB and MIZ expressed an interest in acquiring a group leader with an expertise in this area. It was also agreed that the new group leader should have their own scientific program, rather than just being a "high level" technician for genomic projects of interest to the two institutes. Possible areas of interest could be de novo sequencing and assembly, RNAseq, CHIPseq, sequencing of modified or ancient DNA. An international competition for a joint group leader was advertised and will be resolved in the fall of 2013.

International Cooperation

Max Planck Society

The cooperation started in 2001 as an initiative of the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN). According to the agreement, Junior Research Group, with Dr. Matthias Bochtler as Lab Leader, was funded by the MPG and hosted at the IIMCB. Dr. Bochtler's term at the IIMCB under MPG funding lasted full nine years and became a great scientific success. After a short break in the UK he returned to Poland and currently holds a cross-appointment

at the IIMCB and the Institute of Biochemistry and Biophysics PAN in Warsaw as a full professor.

The Laboratory of Cell Cortex Mechanics MPG/PAN headed by Dr. Ewa Paluch, a twin lab of Matthias Bochtler's MPG/PAN laboratory, was established in February 2006. The equipment and running costs of the lab, including personnel, were covered by Polish funds, but the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG), being a host for this laboratory, was responsible for local operational costs, maintenance, and administrative support. Dr. Ewa Paluch's group focused on biochemical and physical mechanisms of cell shape and deformations. The research, funded mainly by the Polish Ministry of Science and Education, concentrated on movements of the actomyosin cortex and, in particular, on the involvement of spontaneous cortical ruptures and flows in cell division. The group's most spectacular achievements to date are: a paper published in "Nature"and the recently received ERC grant. Currently, Dr. Paluch is relocating her research activities to the University College London under an arrangement whereby she formally remains an IIMCB employee on a leave of absence for the duration of the ERC project and still retains the use of part of our research equipment,which allows research at the new location to commence without undue delay.

In March 2012, a cooperation agreement was signed between IIMCB and the Max-Planck Society (MPG). The agreement concerns the establishment of two Max Planck/ IIMCB Research Groups, one in IIMCB, the other one in the Max-Planck Institute of Heart and Lung Research (MPI-HLR) in Bad Nauheim. Each of the parties will finance a research group with its own budget. The position at Bad Nauheim has already been filled by Dr. Michael Potente, for the position in Warsaw competition is underway. The IIMCB research group is to focus on studies using the zebrafish model; the fish colony has already been established and started its activity as a core facility. The new research laboratory is planned as the first unit in Poland working on zebrafish as a model of pathomechanisms of various human diseases. The existing significant experience of MPG in this area should greatly facilitate the rapid launch and research progress of such a unit.

Institute of Molecular Biology and Genetics, Kiev, Ukraine

Since many years IIMCB collaborates with the Institute of Molecular Biology and Genetics (IMBG) in Kiev, Ukraine. Scientists from both institutions used to meet regularly at the Polish-Ukrainian Parnas Conferences. This cooperation was intensified in 2008 on the initiative of Prof. Kuźnicki who visited IMBG to share experience in managing of a modern research institute and in participating in the EU Framework Programmes, namely FP7 Research Potential scheme. In the following year, IIMCB organized a Polish-Ukrainian scientific conference accompanied by the HEALTH-PROT kick-off meeting. IMBG director, Prof. Anna Elskaya, participated in the meeting of IIMCB's International Advisory Board and IMBG managers had meetings with their IIMCB counterparts. This intense cooperation evolved into shared participation in a three-year COMBIOM project entitled "Strengthening Cooperation in Molecular Biomedicine between EU and Ukraine" (01.12.2011-30.11.2014), supported by FP7 under the ERA-WIDE activity (Integrating Europe's neighbours into the ERA). Apart from the IMBG (coordinator) and IIMCB, the third partner involved is the Institute Gustave-Roussy (IGR) from France.

IIMCB's role in COMBIOM project is to support Ukrainian institute by twinning with Ukrainian researchers (Bochtler, Jaworski, Miączyńska), providing training for IMBG researchers and administration staff, and developing IMBG's Biomed Research Strategy (Kuźnicki, Żylicz).

Year 2012 flourished with common activities. In May a group of IIMCB scientists (Bochtler, Jaworski, Miączyńska, Kuźnicki) and administration representatives (Libiszowska, Ogonowski, Szczepanowski) involved in COMBIOM visited IMBG to initiate twinning collaboration, participate in the first IMBG Advisory Board meeting (Kuźnicki) and the project kick-off meeting. Throughout a year young scientists from IMBG visited IIMCB labs to develop common research projects and twin with our scientists (Prof. Lubov Lukash, Dr. Anna Yatsyshyna – Bochtler Lab, Dr. Mykola Dergai – Miączyńska Lab, DmytrMorderer – Jaworski Lab). Also, in fall 2012, IIMCB organized a weekly training on scientific communication for IMBG researchers provided by an expert, Prof. Edward Potworowski. In the same time, Dr. Yanina Mishchuk, COMBIOM manager, visited International Cooperation Unit of IIMCB to receive training and gain experience in European and other grants management.

Fishing for Medicines and their targets using Zebrafish models of human diseases

Coordination and support actions project financed by the 7th Framework Programme of the European Union within the Research Potential scheme

www.fishmed.iimcb.gov.pl

IIMCB's strategic objective is to reach the quality of research and innovative activities of leading research entities in the world. To achieve this level of excellence and increase our innovative potential, we have introduced new research model: zebrafish. The FishMed Centre, supported by the European Union with 3.5 million €, is composed of a Zebrafish Core Facility and research groups using zebrafish in innovative projects aimed at studies of molecular mechanisms of diseases. The EU funding will be used to finance: the employment of twenty scientists, technicians and managers, purchase of state-of-the-art equipment, exchange visits between IIMCB researchers and their European partners, participation in and organization of various events, including these related to innovation and technology transfer.

FishMed project has following objectives:

- Twinning of seven IIMCB groups with excellent European zebrafish centres to develop innovative potential using fish models.
- Development of a Zebrafish Core Facility and creation of a new research group headed by a leader selected through an open international competition.
- Acquisition and upgrading of research equipment for a Zebrafish Core Facility and new zebrafish research laboratory.
- Reinforcement of IIMCB innovation potential with the Bio&Technology Innovations Platform (BioTech-IP).
- Creation of an interactive visibility platform to popularise the FishMed Centre and research with zebrafish models among scientific and non-scientific communities, including promotion of the project's innovative results.

Twinning and research

The FishMed Centre is a consortium of seven groups from IIMCB and six European institutions, including the Max Planck Institute for Heart and Lung Research (MPI-HLR) as a strategic partner. The twinning partners were chosen based on their expertise in research using zebrafish models, excellent publication records, and compatibility with the scientific interests of the FishMed Centre groups at IIMCB. European partners will share their zebrafish models and expertise related to fish research and thanks to which we will be able to quickly implement our scientific plans. The twinning will allow us to smoothly pass the initial phase of accommodating a new experimental model and quickly focus on cutting-edge research that is likely to lead to innovations.

Twinning partners and research projects:

Matthias Bochtler, Laboratory of Structural Biology, IIMCB and **Carl-Philipp Heisenberg**, the Austrian Institute of Science and Technology (IST), Klosterneuburg, Austria. ***DNA methylation and demethylation in zebrafish.***

Janusz Bujnicki, Laboratory of Bioinformatics and Protein Engineering, IIMCB and **Thomas Braun**, Max-Planck-Institute for Heart and Lung Research, Bad-Nauheim, Germany. ***The development and application of bioinformatics software for the prediction of the pathogenic effects of mutations in protein- and RNA-coding loci.***

Agnieszka Chacińska, Laboratory of Mitochondrial Biogenesis, IIMCB and **Didier Stainier**, Max-Planck-Institute for Heart and Lung Research, Bad-Nauheim, Germany. ***The role of protein import pathways in zebrafish development.***

Jacek Jaworski, Laboratory of Molecular and Cellular Neurobiology, IIMCB and **William Harris**, University of Cambridge, United Kingdom. ***Development of the zebrafish visual system as an in vivo model to study zTOR function and dysfunction in neurons.***

Jacek Kuźnicki, Laboratory of Neurodegeneration, IIMCB and **Oliver Bandmann**, MRC at the University of Sheffield, United Kingdom. ***The mechanism of calcium perturbation in PINK1 mutant of zebrafish, a model of Parkinson's disease.***

Marta Miączyńska, Laboratory of Cell Biology, IIMCB and **Marcos Gonzalez-Gaitan**, Department of Biochemistry, University of Geneva, Switzerland. ***The role of endocytic proteins in signalling and transcriptional regulation in zebrafish.***

Maciej Żylicz, Department of Molecular Biology, IIMCB and **Ewa Snaar-Jagalska**, Department of Molecular Cell Biology, Institute of Biology, Leiden University, The Netherlands. ***The Heat Shock Protein network and p53 response in zebrafish.***

Zebrafish Core Facility

One of the objectives of the FishMed project is development of the Zebrafish Core Facility. This fully licensed facility is run by Dr. Małgorzata Wiweger and 3 technicians. External veterinary inspection is done on weekly bases. The facility which was

opened in November 2012, comprises a water plant, a stand-alone unit (quarantine) and the main system manufactured by Tecniplast. The system can hold approximately 6000 of adult fish but further extension is planned. Currently, 25 different lines of zebrafish are being bred in 131 tanks. In the spring 2013 IIMCB's Zebrafish Core Facility should be fully operational and ready to supply fish and expertise to the internal and external users (for more information see page 83).

New research group

A new group leader will be selected during an open international competition organised jointly with MPI-HLR in Bad Nauheim, our strategic partner for the creation and development of the FishMed Centre. The group located at IIMCB will have full access to MPI-HLR equipment, animal facilities, and genetic modification techniques for zebrafish and mice. The expertise of the new group leader and his/her staff will further help us achieve a critical mass for the development of competitive and innovative zebrafish projects.

Research equipment

IIMCB laboratories are equipped with state-of-the-art scientific equipment. However, several large pieces of sophisticated equipment will be needed for zebrafish-related research, such as a fluorescence microscope system for time-lapse imaging of zebrafish (e.g. single-plane illumination fluorescence microscope), light and fluorescence stereomicroscopes, microinjectors, and a system for the behavioural analysis of larvae and adult zebrafish. Additionally, the Zebrafish Core Facility will be expanded by the purchase of additional pieces of equipment, such as stereomicroscopes and laboratory incubators.

Innovations Platform

In response to IIMCB growing potential, a separate unit was established in March 2010 to deal with applied technology generated at IIMCB, referred to as the Bio&Technology Innovations Platform (BioTech-IP). Presently, Biotech-IP's aim is to find, protect, and commercialise projects that display market potential. Within the RegPot funding, BioTech-IP will deal with applied R&D outcomes of the FishMed projects that are crucial for securing new products and technologies that may stem from them. In the long-term process of technology transfer, BioTech-IP will be assisted by experienced specialists with international expertise in the legal and financial aspects of dealing with technology, such as technology assessment, patent law, market research, license agreement drafting, etc. Such experts will complement the BioTech-IP staff, who will then be able to continue professional support for FishMed Centre scientists to identify the innovation potential of their research, help protect their IP rights, locate the funds needed to move to the commercial phase, and secure long-term gains for society. We plan up to three filed patent applications, several reports on technology evaluations or license agreements, business-to-science brunches, and two training courses on IPR and related issues delivered to Centre scientists to further foster their interest and focus on applied research.

FishMed visibility

Promotional, dissemination, and popularization activities performed by IIMCB need professional coordination and adjustments to rapidly developing needs. This task will be achieved by newly employed public relations manager. We plan to create an interactive visibility platform, a set of integrated activities that focus on the popularisation of the FishMed Centre and research on zebrafish models among scientific and non-scientific communities, and promoting the project results for commercialisation purposes. The visibility platform will focus on several main tasks: 1/ it will develop cooperation with relevant target groups from outside academia, such as patient organisations, local authorities, and the general public. This initiative will allow for the better identification of societal and market needs, sensitize public authorities to the importance of health research, and increase social awareness and enthusiasm for research activities; 2/ it will create a discussion forum for the Polish scientific community on the application of zebrafish models for studies of human diseases as an alternative to mammalian models. We will introduce the concept of zebrafish research to the local scientific community, become a local source of information, and create discussion, knowledge, and intellectual exchange on new research models; 3/ it will disseminate the scientific results of the FishMed Centre through publications, lectures, and posters.

Management

FishMed project is coordinated by Professor Jacek Kuźnicki, the author of the idea of setting up the FishMed Centre at IIMCB. He is advised by the International Advisory Board and the FishMed Committee. The project management team consists of a project manager, Dr. Urszula Bialek-Wyrzykowska and leaders of workpackages: Prof. Jacek Kuźnicki (WP1), Prof. Michał Witt (WP2), Prof. Alicja Żylicz (WP3), Magdalena Powierża (WP4), Dorota Libiszowska (WP5) and Dr. Urszula Białek-Wyrzykowska (WP6 and WP7).

Kick-off meeting

On April 12, 2013 IIMCB organizes the FishMed kick-off meeting, an event consisting of a scientific symposium and a project meeting. The European Commission will be represented by a policy officer Grzegorz Ambroziewicz. All European partners involved in the project will participate in the meeting and will deliver lectures during the scientific symposium (see a poster on the inner cover). To this open part of the event we invited scientists, representatives of the institutions responsible for science policy in Poland, members of International Advisory Board to IIMCB, representatives of patient organizations, media and many more. During the closed project meeting the issues related project implementation and management will be discussed.

Current developments

The most important achievements of the project since its launch in December 2012, are fully operational Zebrafish Core Facility (see page 83) and the employment of staff: four experienced postdoctoral researchers, two technical assistants, leader and technicians of the core facility, PR and project managers. On April 19 Prof. Robert Huber, the Nobel Prize laureate, will inaugurate an open seminar series – a discussion forum on the usage of zebrafish for studies of human diseases.

Lab Leader Competitions

International competitions for lab leaders' competitions are considered at IIMCB as essential mechanism for ensuring influx of talented young researchers to the Institute. This procedure is mandatory, in our opinion, unquestionably leading to a continuous increase of IIMCB scientific level. As a rule each competition is advertised in internationally visible media, both printed (Nature) and electronic (NatureJobs, Euraxess, IIMCB web page).

The applicants are initially screened formally at the Institute, and later, get evaluated by the Selection Committee, made up of several members of the International Advisory Board (IAB). Shortlisted candidates with the highest score receive invitations to give a presentation in a symposium run publicly with the participation of IAB members. The final recommendation is made by IAB and passed to the Director, who comes with the binding decision.

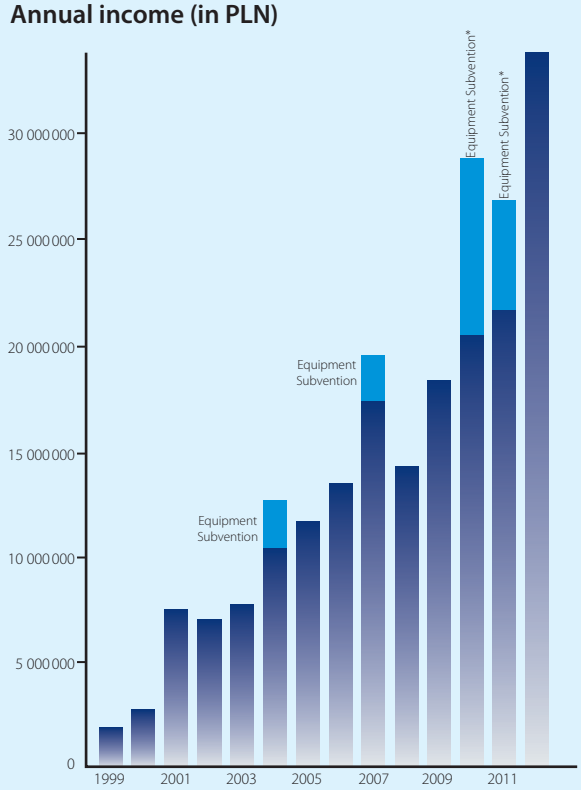
We believe that the sharp selection criteria and objective and completely factual recruitment process of lab leaders is key to the success of such an institution as IIMCB. It is the starting point for dynamic growth, opening new lines of research and introduction of modern technology at the Institute. This way of recruitment enables hiring of the most talented researchers - providing them with appropriate conditions of development in IIMCB often becomes the first step to independent, international scientific careers.

Competition	Year	Number of candidates	**Winners employed at IIMCB**
I	1998	6	**Jarosław Dastych**
II	1999	3	**Maciej Żylicz**
III	2000	6	**Michał Hetman**
IV[1)]	2000	7	**Matthias Bochtler, Leszek Rychlewski**
V	2002	9	**Janusz M. Bujnicki, Sławomir Filipek**
VI[2)]	2002	9	**–**
VII	2003	18	**Marta Miączyńska**
VIII[3)]	2004	26	**–**
IX	2005	26	**Jacek Jaworski**
X[1)]	2005	17	**Ewa Paluch**
XI	2006	25	**Marcin Nowotny**
XII[3)]	2007	16	**–**
XIII	2008	14	**Agnieszka Chacińska**
XIV[2)]	2010	20	**–**
$XV^{3,4)}_{ab}$	2012	18 & 15	**–**
XVI[5)]	2013	?	**pending**

[1)] these competitions fulfilled the MPG/PAN agreement
[2)] no result
[3)] the winner did not accept the offer
[4)] this competition fulfilled the MPG/IIMCB agreement
[5)] this competition fullfils the Museum and Institute of Zoology PAN/IIMCB agreement

Diversity of Funding IIMCB'2012

Annual income (in PLN)



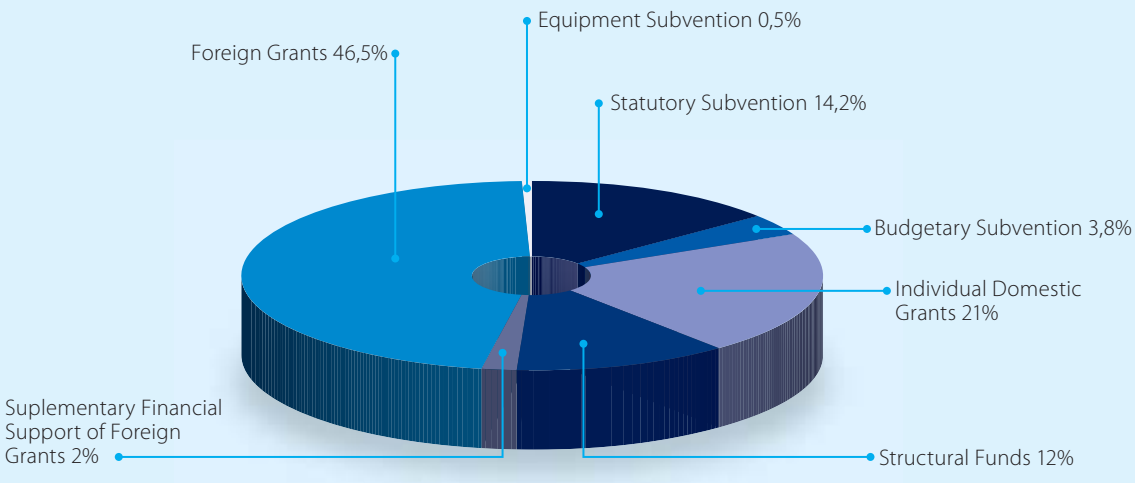
* 5,000,000 PLN from Structural Project CePT (Ministerial)

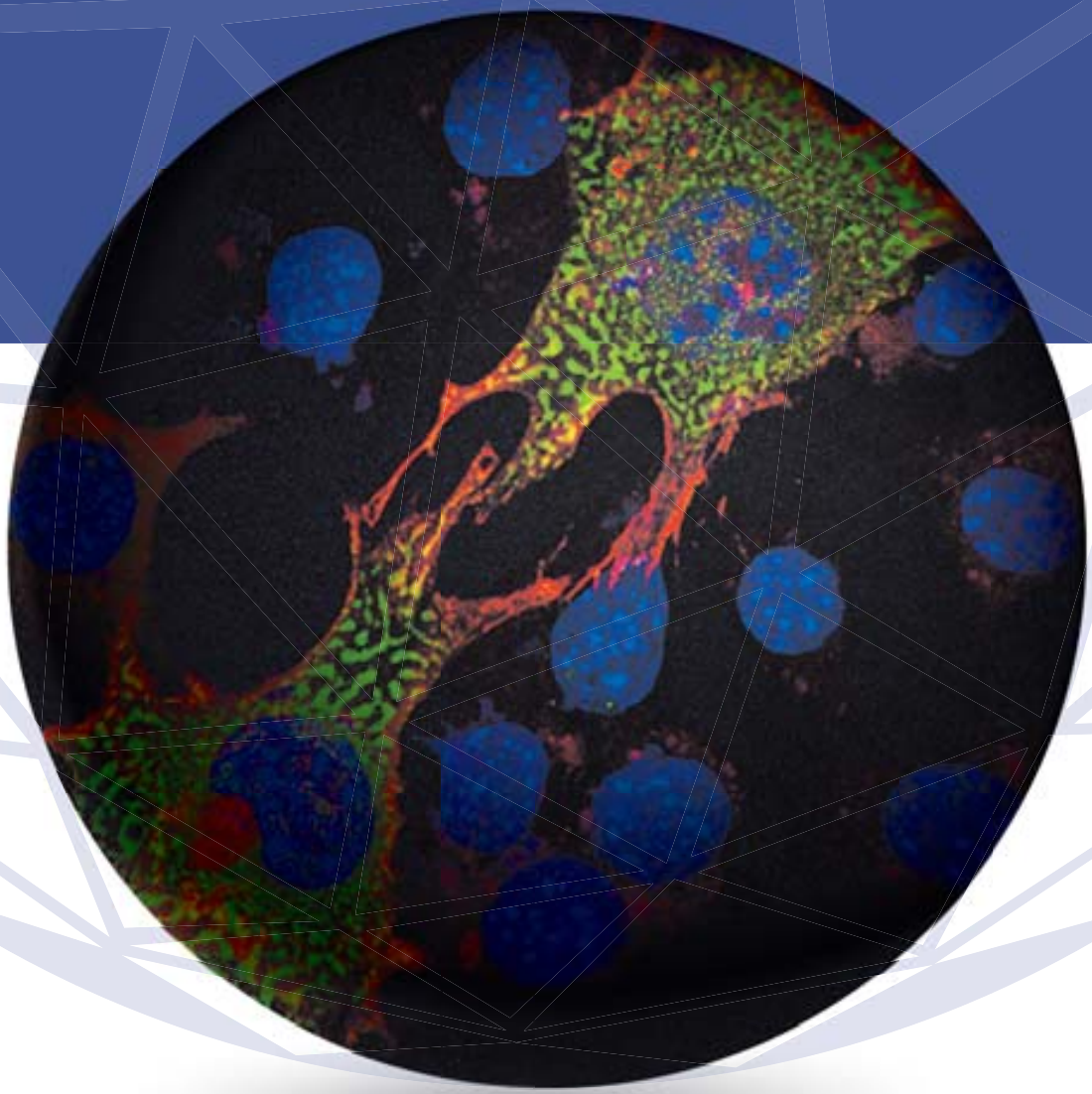
Sources of Funding	amounts in PLN	amounts in EUR[1]
Statutory Subvention	4 752 598	1 162 516
Budgetary Subvention	1 274 000	311 629
Individual Domestic Grants	7 017 119	1 716 432
Structural Funds	3 992 064	976 485
Supplementary Financial Support of Foreign Grants	672 971	164 613
Foreign Grants	15 539 666	3 801 102
Equipment Subvention	150 000	36 691
Total	**33 398 418**	**8 169 468**

[1] 1EUR - 4,0882 @ 31st Dec'2012

Profit & loss statement

	amounts in PLN
A. net revenue on sales and equivalents*	23 419 006
B. operational activity costs:	23 890 452
Depreciation (equipment)	823 834
Research materials	6 396 135
Utilities	412 339
Services	2 657 008
Fees and taxes	407 107
Salaries and wages	8 945 397
Social and health insurance	2 100 313
Other operational expenses, in this:	2 148 319
business trips	1 120 573
property insurance	28 537
fellowships	971 300
others	27 909
C. other operational income (subventions)	494 545
D. other operational expenses:	3 147
E. financial income (interests):	375 826
F. financial expenses (others):	659
Profit/Loss on business activity (A-B+C-D+E-F) +	**395 118**





"Dancing cells" – MEF cells transfected with plasmids encoding p53 R175H and HA-HSP70. 48 hours post-transfection the cells were fixed and stained with p53 (pseudocolour green) and HA tag (pseudocolour red) specific antibodies (Milena Wiech)

Department of **Molecular Biology**

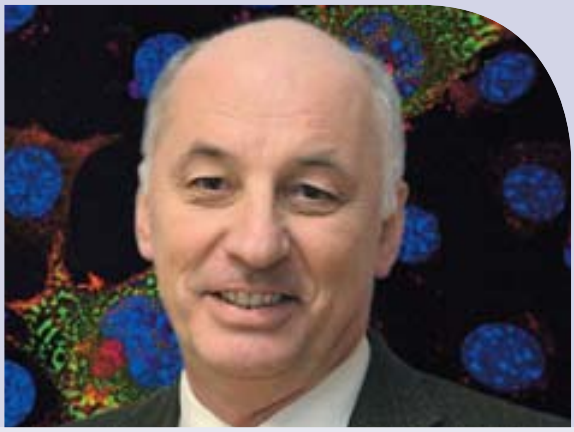
Lab leader: **Maciej Żylicz**, PhD, Professor

Senior Researcher:
Bartosz Wawrzynów, PhD

Junior Researchers:
Marta Małuszek, MSc
Magdalena Pruszko, MSc
Zuzanna Tracz-Gaszewska, MSc
Milena Wiech, MSc

Secretary:
Grażyna Orleańska, MSc

Technician:
Wanda Gocal



Head of Department of Molecular Biology

Maciej Żylicz, PhD, Professor

Degrees

1992	Professor, nomination by the President of the Republic of Poland
1986	DSc Habil in Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
1980	PhD in Biochemistry, Medical University of Gdansk, Poland
1977	MSc in Physics, University of Gdansk, Poland (student of physics and biology)

Postdoctoral Training

1982-1984	Department of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City, Utah, USA, and Department of Biochemistry, Stanford University, Stanford, California, USA
1979-1981	Department of Biochemistry, University of Gdansk, Poland

Professional Employment

2005-Present	President, Executive Director, Foundation for Polish Science
1999-Present	Head, Department of Molecular Biology, IIMCB
1994-1999	Head, Department of Molecular and Cellular Biology, Faculty of Biotechnology, University of Gdansk, Poland
1991-1994	Head, Department of Molecular Biology, University of Gdansk, Poland
1993-1994	Visiting Professor, University of Utah, Medical Center, Institute of Oncology, Salt Lake City, Utah, USA
1990-1993	Vice President, University of Gdansk, Poland
1988-1991	Associate Professor, Department of Molecular Biology, University of Gdansk, Poland
1981-1988	Assistant Professor, Department of Biochemistry, University of Gdansk, Poland

Other Professional Activities

2010-Present	Advisor of the President of the Republic of Poland
2010-Present	Member, ERC Identification Committee (appointed by European Commission)
2010-Present	Chair of Selection Committee, Council of the National Science Center, Poland
2008-2010	Panel Chair, Molecular and Structural Biology and Biochemistry (LS1), ERC
2000-2004	Chair of Biology, Earth Sciences and Environmental Protection Commission, State Committee for Scientific Research, Poland
2000-2001	Chair of Basic Science Commission, State Committee for Scientific Research, Poland

Membership in Scientific Societies, Organizations, and Panels

- European Molecular Biology Organization (EMBO), Member
- Polish Academy of Sciences, Full Member
- German National Academy of Sciences Leopoldina, Member
- Polish Academy of Arts and Sciences, Member
- Academia Europaea, Member
- European Academy of Cancer Research, Member
- American Society of Biochemistry and Molecular Biology, Member
- Advisory Editorial Board, EMBO Journal, EMBO Reports (2004-2008), and IUBMB Life, Member
- EMBO Council (2004-2007), Member
- Selection Committee, EMBO Young Investigator Programme (2001-2003), Member
- European Molecular Biology Conference (2001-2004), Polish delegate
- European Science Foundation Life Science Committee (2003-2005), Polish Delegate
- Selection Committee, Special DFG Programmes (2001-2005), Member
- Max Planck Society, Member of Senate (2012-Present)
- State Committee for Scientific Research (1997-2004), Member

Honors, Prizes and Awards

2013	Doctor Honoris Causa, Jagiellonian University
2011	Doctor Honoris Causa, University of Gdansk
2008	Officer's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)
2007	Doctor Honoris Causa, University of Wrocław
2002	Prime Minister Award for Scientific Achievements
2001	Marchlewski Award, Committee of Biochemistry and Biophysics, Polish Academy of Sciences
1999	Award in biological/medical sciences, Foundation for Polish Science

1996, 2007, 2010 Awards for best biochemistry work performed in Polish laboratories, Polish Biochemical Society
1994 Award from Ministry of Education
1993 Heweliusz Prize for Scientific Achievements (awarded by President of Gdansk)
1990 Award from Polish Academy of Sciences
1986 Individual Award for Scientific Achievements, Polish Academy of Sciences

Doctorates

Liberek K, Skowyra D, Osipiuk J, Banecki B, Wojtkowiak D, Jakóbkiewicz J, Puzewicz J, Barski P, King F, Bućko-Justyna M, Kudła G, Helwak A, Lipiński L, Szymańska Z, Urbański J

Academic Habilitations

Liberek K, Werel W, Marszałek J, Konieczny I, Wawrzynów A, Banecki B, Bieganowski P

Professor Titles Received

Liberek K, Marszalek J, Konieczny I, Wawrzynów A

Publications

Over 80 publications in primary scientific journals, including two papers published in *Cell*, six in *EMBO J*, six in *Proc Natl Acad Sci USA*, and more than 30 in *J Biol Chem*. These papers were cited more than 5500 times with a Hirsch index of H = 40.

Selected publications

- **Wiech M, Olszewski M, Tracz-Gaszewska Z, Wawrzynow B, Zylicz M, Zylicz A**. Molecular Mechanism of Mutant p53 Stabilization: The Role of HSP70 and MDM2. PLoS One, 2012; 7(12):e51426
- Hageman J, van Waarde MA, **Zylicz A**, **Walerych D**, Kampinga HH. The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities. **Biochem J**, 2011; 435:127-142
- **Walerych D, Gutkowska M, Klejman MP, Wawrzynow B, Tracz Z, Wiech M, Zylicz M, Zylicz A**. ATP binding to Hsp90 is sufficient for effective chaperoning of p53 protein. J Biol Chem, 2010; 285:32020-8
- Zubrienė A, **Gutkowska M**, Matulienė J, Chaleckis R, Michailovienė V, Voroncova A, Venclovas C, **Zylicz A, Zylicz M**, Matulis D. Thermodynamics of radicicol binding to human Hsp90 alpha and beta isoforms. Biophys Chem, 2010; 152:153-163
- **Zurawska A, Urbanski J**, Matulienė J, Baraniak J, **Klejman MP**, Filipek S, Matulis D, **Bieganowski P**. Mutations that increase both Hsp90 ATPase activity in vitro and Hsp90 drug resistance in vivo. Biochim Biophys Acta – Mol Cell Res, 2010; 1803:575-583
- Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, **Zylicz A**, Knudsen J, Sandhoff K, Arenz C, Kinnunen PK, Nylandsted J, Jäättelä M. Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. Nature, 2010; 463:549-553
- **Walerych D, Olszewski MB, Gutkowska M, Helwak A, Zylicz M, Zylicz A**. Hsp70 molecular chaperones are required to support p53 tumor suppressor activity under stress conditions. Oncogene, 2009; 28:4284-94
- Narayan V, Eckert M, **Zylicz A, Zylicz M**, Ball KL. Cooperative regulation of the interferon regulatory factor-1 tumor suppressor protein by core components of the molecular chaperone machinery. J Biol Chem, 2009; 284:25889-99
- Wawrzynow B, Pettersson S, **Zylicz A**, Bramham J, Worrall E, Hupp TR, Ball KL. A function for the RING finger domain in the allosteric control of MDM2 conformation and activity. J Biol Chem, 2009; 284:11517-30
- **Szymanska Z, Zylicz M**. Mathematical modeling of heat shock protein synthesis in response to temperature change. J Theor Biol, 2009; 259:562-569
- **Szymanska Z, Urbanski J**, Marciniak-Czochra A. Mathematical modelling of the influence of heat shock proteins on cancer invasion of tissue. J Math Biol, 2009; 58:819-44
- **Zurawska A, Urbanski J, Bieganowski P**. Hsp90n - An accidental product of a fortuitous chromosomal translocation rather than a regular Hsp90 family member of human proteome. Biochim Biophys Acta, 2008; 1784:1844-6
- Stevens C, Pettersson S, **Wawrzynow B**, Wallace M, Ball K, **Zylicz A**, Hupp TR. ATP stimulates MDM2-mediated inhibition of the DNA-binding function of E2F1. FEBS J, 2008; 275:4875-86
- **Wawrzynow B, Zylicz A**, Wallace M, Hupp T, **Zylicz M**. MDM2 chaperones the p53 tumor suppressor. J Biol Chem, 2007; 282:32603-12
- Issat T, Nowis D, Legat M, Makowski M, **Klejman MP, Urbanski J**, Skierski J, Koronkiewicz M, Stoklosa T, Brzezinska A, Bil J, Gietka J, Jakobisiak M, Golab J. Potentiated antitumor effects of the combination treatment with statins and pamidronate *in vitro* and *in vivo*. Int J Oncol, 2007; 30:1413-25
- **Kudla G, Lipinski L, Caffin F, Helwak A, Zylicz M**. High guanine and cytosine content increases mRNA levels in mammalian cells. PLoS Biology, 2006; 4:0933-42
- **Walerych D, Kudla G, Gutkowska M, Wawrzynow B**, Muller L, **King FW, Helwak A, Boros J, Zylicz A, Zylicz M**. Hsp90 chaperones wild-type p53 tumor suppressor protein. J Biol Chem, 2004; 279: 48836-45
- Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, **Zylicz M**, Jakobkiewicz-Banecka J, Kobierska-Gulida G, Szymanowska A, Skokowski J, Roessner A, Schneider-Stock R. MDM2 gene amplification: a new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC) Lung Cancer, 2004; 43:285-295
- **Kudla G, Helwak A, Lipinski L**. Gene conversion and GCcontent evolution in mammalian Hsp70. Mol Biol Evol, 2004; 21:1438-44
- **Zylicz M, King FW, Wawrzynow A**. Hsp70 interactions with the p53 tumour suppressor protein. EMBO J, 2001; 20:4634-8
- **King FW, Wawrzynow A**, Hohfeld J, **Zylicz M**. Cochaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. EMBO J, 2001; 20:6297-305.

Summary of work

The research conducted in the Department of Molecular Biology mainly focuses on the activity of molecular chaperones in mammalian cells, including cell transformation. Using highly purified recombinant human proteins, we previously identified intermediate reactions that led to the assembly of molecular chaperone complexes with the wildtype or mutant p53 tumor suppressor protein. We also demonstrated that the heat shock protein 90 (HSP90) molecular chaperone was required for the binding of wildtype p53 to the promoter sequences under a physiological temperature of 37ºC and that chaperoning activity was adenosine triphosphate (ATP)-dependent. We recently provided *in vivo* evidence that HSP90 and HSP70/HSPA chaperone machines were required for the proper folding of wildtype p53, its specific binding to chromatin, and the transcription of p53-dependent genes (Walerych et al., *Oncogene*, 2009). We showed that molecular chaperones in human cells transfected with wildtype *TP53*, *HSP90*, and *HSP70* maintained the native p53 conformation under heat-shock conditions (42ºC) and assisted with refolding p53 at 37ºC during the recovery from heat shock. We also demonstrated that the interaction between wildtype p53 and the *WAF1* promoter in cells was sensitive to HSP70 and HSP90 inhibition at 37ºC and further decreased upon heat shock. The influence of chaperones on the binding of p53 to the *WAF1* promoter sequence was confirmed *in vitro* using highly purified proteins. HSP90 stabilized the binding of p53 to the promoter sequence at 37ºC, whereas the requirement for the HSP70-HSP40 system and its cooperation with HSP90 increased under heat shock conditions. The Hop co-chaperone additionally stimulated these reactions. Interestingly, the combination of HSP90 and HSP70-HSP40 allowed for a limited *in vitro* restoration of DNA binding activity by the p53 oncogenic variant R249S and affected its conformation in cells. Our results indicated that both HSP90 and HSP70 were required for the chaperoning of wildtype and R249S p53, especially under stress conditions (Walerych et al., *Oncogene*, 2009).

We also elucidated the role of the adenine nucleotide in the HSP90 chaperone cycle by taking advantage of a unique *in vitro* assay that measures the HSP90-dependent binding of p53 to the promoter sequence (Walerych et al., *J Biol Chem*, 2010). E42A and D88N HSP90β variants bound but did not hydrolyze ATP, whereas E42A increased and D88N decreased ATP affinity compared with wildtype HSP90β. Nevertheless, both of these mutants interacted with wildtype p53 with similar affinity. Surprisingly, in the case of wildtype and E42A HSP90β, the presence of ATP stimulated the dissociation of HSP90-p53 complexes and resulted in the binding of p53 to the promoter sequence. D88N HSP90β is not efficient in either of these reactions. Using a trap version of the GroEL chaperonin, which irreversibly captures unfolded proteins, we showed that the action of the HSP90 chaperone on wildtype p53 resulted in a partial unfolding of the substrate. The ATP-dependent dissociation of the p53-HSP90 complex allowed further folding of the p53 protein to an active conformation that was able to bind to the promoter sequence. Further supporting these results, the overproduction of wildtype or E42A HSP90β stimulated transcription from the *WAF1* gene promoter in H1299 cells. Altogether, our research indicated that the binding of ATP to HSP90β was a sufficient step for effective wildtype p53 client protein chaperoning (Walerych et al., *J Biol Chem*, 2010).

Heat shock protein 70 (HSP70/HSPA1) is an evolutionarily highly conserved molecular chaperone that promotes the survival of stressed cells by inhibiting lysosomal membrane permeabilization, a hallmark of stress-induced cell death. Clues to its molecular mechanism of action may lie in the recently reported stress- and cancer-associated translocation of a small portion of HSP70 to the lysosomal compartment. Prof. Marja Jaattela's laboratory at the Denmark Cancer Institute, in collaboration with our department, showed that HSP70 stabilized lysosomes by binding to endolysosomal anionic phospholipid bis(monoacylglycero)phosphate (BMP), an essential cofactor for lysosomal sphingomyelin metabolism (Kirkegaard et al., *Nature*, 2010). In acidic environments, HSP70 binds with high affinity and specificity to BMP, thereby facilitating the BMP binding and activity of acid sphingomyelinase (ASM). Inhibition of the HSP70-BMP interaction by BMP antibodies or a point mutation in HSP70 (Trp90Phe) and the pharmacological and genetic inhibition of ASM effectively reversed the HSP70-mediated stabilization of lysosomes. Notably, the reduced ASM activity in cells from patients with Niemann-Pick disease (NPD) A and B (i.e., severe lysosomal storage disorders caused by mutations in the sphingomyelin phosphodiesterase 1 [*SMPD1*] gene that encodes ASM) was also associated with a marked decrease in lysosomal stability, and this phenotype could be effectively corrected by treatment with recombinant HSP70. Altogether, these data open exciting possibilities for the development of new treatments for lysosomal storage disorders and cancer with compounds that enter the lysosomal lumen through the endocytic delivery pathway (Kirkegaard et al., *Nature*, 2010).

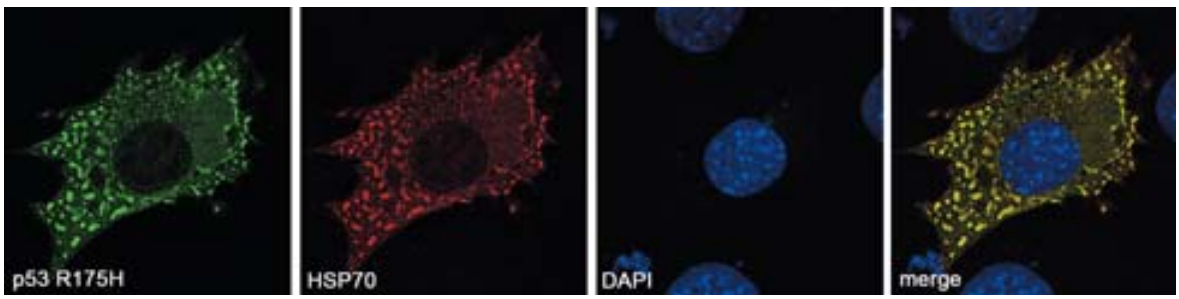


Fig. 1. Colocalization of overproduced HSP70 with mutated p53 R175H in MEF cells. MEF cell were transfected with plasmids encoding p53 R175H and HA-HSP70. 48 hours post-transfection the cells were fixed and stained with p53 (pseudocolour green) and HA tag (pseudocolour red) specific antibodies.

We discovered that MDM2, in addition to its E3-ubiquitin ligase activity, exhibited molecular chaperone activity and demonstrated that a MDM2 mutant protein that is defective in ATP binding (K454A) lacked chaperone activity both *in vivo* and *in vitro*. Wildtype *MDM2* coexpressed with wildtype *p53* stimulated efficient p53 protein folding *in vivo*, and this effect was abrogated with an ATP binding-defective form of MDM2 (Wawrzynow et al., *J Biol Chem*, 2007).

Recently, in collaboration with Prof. Kathryn Ball at the University of Edinburgh, we showed that the binding affinity of MDM2's hydrophobic pocket could be regulated through the RING finger domain and that increases in pocket affinity were reflected by a gain in MDM2 transrepressor activity (Wawrzynow et al., *J Biol Chem*, 2009). Thus, mutations within the RING domain that affect zinc coordination but not mutations that inhibit ATP binding produce MDM2 proteins that have a higher affinity for the BOX-I transactivation domain of p53 and a reduced affinity for p53 transrepression. An allosteric model of the regulation of the hydrophobic pocket was supported by differences in protein conformation and pocket accessibility between wildtype and RING domain mutant MDM2 proteins. Additionally, the data demonstrated that the complex relationship between different domains of MDM2 could impact the efficacy of anticancer drugs directed toward its hydrophobic pocket (Wawrzynow et al., *J Biol Chem*, 2009).

Numerous p53 missense mutations possess gain-of-function activities. Studies in mouse models have demonstrated that the stabilization of p53 R172H (R175H in humans) mutant protein by currently unknown factors is a prerequisite for its oncogenic gain-of-function phenotype, such as tumor progression and metastasis. Recently, we showed that the MDM2-dependent ubiquitination and degradation of p53 R175H mutant protein in mouse embryonic fibroblasts was partially inhibited by increasing concentration of HSP70/HSPA1-A. These phenomena correlated well with the appearance of HSP70-dependent folding intermediates in the form of dynamic cytoplasmic spots that contained aggregate-prone p53 R175H and several molecular chaperones (Fig. 1). We propose that a transient but recurrent interaction with HSP70 may lead to an increase in mutant p53 protein half-life. In the presence of MDM2, these pseudoaggregates can form stable amyloid-like structures that occasionally merge into an aggresome Interestingly, the formation of folding intermediates was not observed in the presence of HSC70/HSPA8, the dominant-negative K71S variant of HSP70, or an HSP70 inhibitor. In cancer cells, where endogenous HSP70 levels are already elevated, mutant p53 protein formed nuclear aggregates without the addition of exogenous HSP70. Aggregates that contained p53 were also visible under conditions in which p53 was partially unfolded (i.e., 37°C for temperature-sensitive variant p53 V143A and 42°C for wildtype p53). The refolding kinetics of p53 indicated that HSP70 caused transient exposure of the p53 aggregate-prone domains. We propose that the formation of HSP70- and MDM2-dependent protein coaggregates in tumors with high levels of these two proteins could be one of the mechanisms by which mutant p53 is stabilized.

Our results suggest that HSP70 molecular chaperone binds and partially unfolds p53. Upon ATP-dependent release of HSP70 from the complex with p53, part of the unfolded p53 protein, with the help of MDM2, is captured in the aggregation-prone conformation (Fig. 2). Moreover, the sequestration of p73 tumor suppressor protein by these nuclear aggregates may lead to gain-of-function phenotypes (Wiech et al., *PLoS One*, 2012).

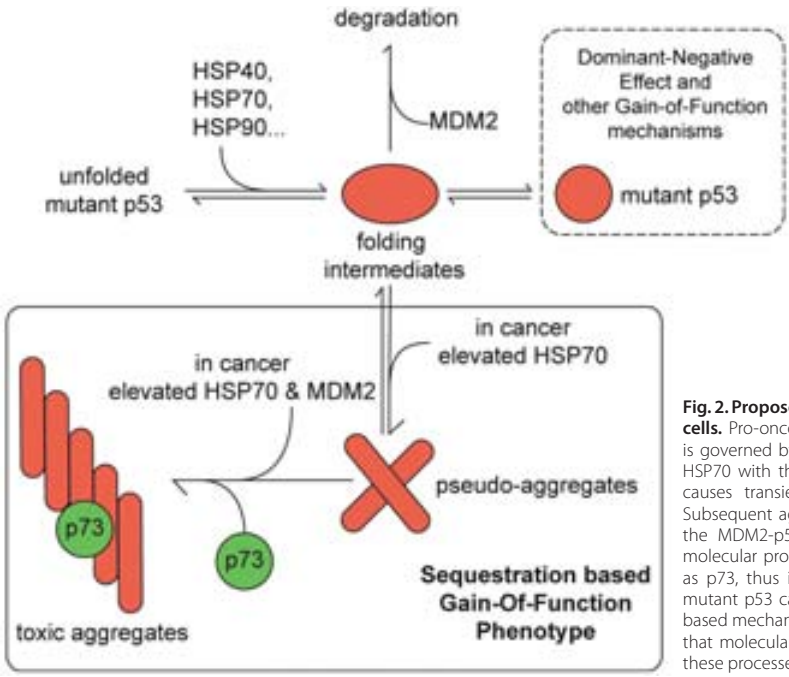
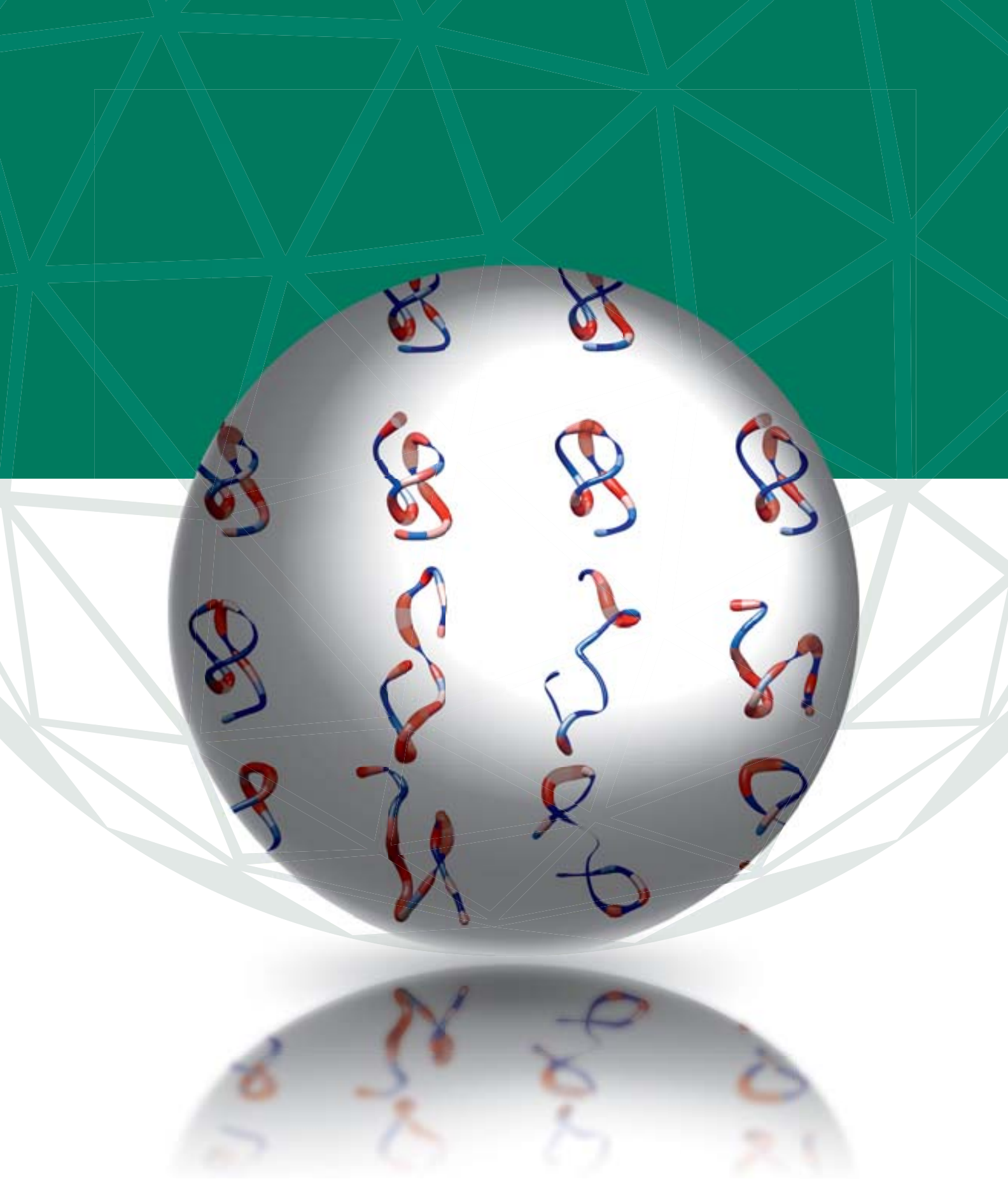


Fig. 2. Proposed model for stabilization of mutant p53 in cancer cells. Pro-oncogenic, gain-of-function phenotype of mutant p53 is governed by HSP70 and MDM2 levels. Recurrent interaction of HSP70 with the p53 polypeptide, in an ATP dependent manner, causes transient exposure of its aggregate prone domain(s). Subsequent aggregation of mutant p53 is further augmented by the MDM2-p53 allosteric interaction. This dynamic, irreversible molecular process can sequester other tumour suppressors, such as p73, thus inhibiting their activity. Pro-oncogenic activities of mutant p53 can be manifested through other, non-sequestration based mechanisms as depicted. We cannot exclude the possibility that molecular chaperones, including HSP70, are also involved in these processes.



Models of the viral RNA pseudoknot (crystal structure: 1l2x.pdb) visualized according to the predicted accuracy (accurate: blue, thin tubes, inaccurate: red, thick tubes). Graphics and data: Przemyslaw Gierski, Marcin Magnus, Michal Boniecki.

Laboratory of **Bioinformatics and Protein Engineering**

Lab leader: **Janusz M. Bujnicki**, PhD, Professor

Postdoctoral Fellows:
Michał Boniecki, PhD; Grzegorz Chojnowski, PhD; Stanisław Dunin-Horkawicz, PhD; Bogusław Kluge, PhD; Grzegorz Łach, PhD; Martyna Nowacka, PhD; Elżbieta Purta, PhD; Izabela Rutkowska-Włodarczyk, PhD (until June 2012); Krzysztof J. Skowronek, PhD (part-time); Tomasz Sołtysiński, PhD (until March 2012); Tomasz Waleń, PhD

Junior Researchers:
Astha, MSc; Magdalena Byszewska, MSc; Ilona Domagała, MSc; Dawid Głow, MSc; Jakub Jopek, MSc (extramural); Katarzyna H. Kamińska, MSc (until November 2012); Łukasz Kozłowski, MSc; Małgorzata Kurkowska (Durawa), MSc; Magdalena Machnicka (Mika), MSc; Marcin Magnus, MSc; Dorota Matelska, MSc; Shamba Sankar Mondal, Msc; Anna Olchowik, MSc; Anna Philips, MSc (until June 2012); Dariusz Pianka, MSc (until October 2012); Paweł Piątkowski, MSc; Michał J. Piętal, MSc (extramural); Katarzyna Poleszak, MSc (until April 2012); Wojciech Potrzebowski, MSc (until June 2012); Juliusz Stasiewicz, Agata Sulej (Kamaszewska), MSc (until September 2012); MSc; Krzysztof Szczepaniak, MSc; Irina Tuszyńska, MSc; Maria Werner, MSc

Research Technicans:
Justyna Czarnecka, PhD;
Sylwia Panek, MSc

Undergraduate Students:
Albert Bogdanowicz, BSc;
Witold Januszewski, BSc;
Kama Wójcik, BSc

Office Manager:
Agnieszka Faliszewska, MSc

Computer Administrators:
Tomasz Jarzynka (part-time); Jan Kogut, BSc (part-time); Łukasz Munio

Head of Laboratory of Bioinformatics and Protein Engineering

Janusz Bujnicki, PhD, Professor

Degrees

2009	Professor of Biological Sciences, nomination by the President of the Republic of Poland
2005	DSc Habil in Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
2001	PhD in Biology, University of Warsaw, Faculty of Biology, Poland
1998	MSc in Microbiology, University of Warsaw, Faculty of Biology, Poland

Professional Experience

2002-Present	Head, Laboratory of Bioinformatics and Protein Engineering, IIMCB
2006-Present	Visiting Associate Professor, Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland
2004-2006	Assistant Professor, Bioinformatics Laboratory, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland
2001-2002	Group Leader, Molecular Evolution Research Group, Laboratory of Bioinformatics, IIMCB
2001	Visiting Scientist, Computational Biology Branch, National Center for Biotechnology Information, National Institutes of Health, Bethesda, Maryland, USA (with Dr. E.V. Koonin)
1999-2000	Research Scientist, Bioinformatics Laboratory, IIMCB (with Dr. L. Rychlewski)
1998-2000	Senior Research Assistant, Molecular Biology Research Program, Henry Ford Health System, Detroit, Michigan, USA (with Dr. L.C. Lutter)

Membership in Scientific Societies, Organizations, and Panels

- Polish Bioinformatics Society, PTBI (founding member, Vice-President 2007-2010, President 2011- Present)
- Society of Bioinformatics in Northern Europe (SocBiN) (board member, 2004-Present)
- Member, International Society for Computational Biology
- Member, RNA Society
- Executive Editor, *Nucleic Acids Research* (2013-Present)
- Series Editor, *Nucleic Acids and Molecular Biology* (Springer Verlag, 2009-Present)
- Deputy Section Editor, *BMC Bioinformatics* (2010-Present)
- Editorial Board, *Nucleic Acids Research* (2005-Present), *Advances in Bioinformatics* (2008-2011), *Journal of Applied Genetics* (2004-Present), *Database Journal* (2008-Present), *Journal of Nucleic Acids* (2008-Present)
- Academy of Young Scientists, Polish Academy of Sciences, AMU-PAN (elected in 2011)

Awards

2012	Award for Outstanding Research Achievements, Ministry of Science and Higher Education
2011	Adam Mickiewicz University Rector Special Award for Top Performance in Publishing High Impact Research Articles in 2010
2010	ERC Starting Grant (2011-2015)
2009	Fellowship for Outstanding Young Scientists, Ministry of Science and Higher Education
2009	Award for Research Achievements, Ministry of Science and Higher Education (Individual work)
2008	Adam Mickiewicz University Rector Award for Research Achievements (Individual work)
2006	Award from Prime Minister for habilitation thesis
2006	Young Researcher Award in Structural and Evolutionary Biology, Visegrad Group Academies of Sciences
2003, 2004	Fellowship for Young Scientists, Foundation for Polish Science
2002	EMBO/Howard Hughes Medical Institute Young Investigator Program Award
2002	Award for best Polish genetics-related publication in 2001 (*Trends Biochem Sci* 2001, Jan, 26[1]:9-11), Polish Society of Genetics
2001	Award for best Polish publication on nucleic acid biochemistry in 2000 (*FASEB J* 2000, Nov, 14[14]:2365-2368), Polish Biochemical Society

Doctorates

Zylicz-Stachula A, Chmiel A, Cymerman I, Czerwoniec A, Gajda M, Pawlowski M, Sasin-Kurowska J, Kosinski J, Obarska-Kosinska A, Pawlak S, Purta E, Tkaczuk K, Koscinski L, Rother M, Potrzebowski W

Publications in 2012

- **Kozlowski LP, Bujnicki JM**. MetaDisorder: A meta-server for the prediction of intrinsic disorder in proteins. BMC Bioinformatics, 2012; 13:111
- Kasprzak JM, Czerwoniec A, **Bujnicki JM**. Molecular evolution of dihydrouridine synthases. BMC Bioinformatics, 2012; 13:153
- **Korneta I, Bujnicki JM**. Intrinsic disorder in the human spliceosomal proteome. PLoS Comput Biol, 2012; 8(8): e1002641
- Kusio-Kobialka M, Wolanin K, Podszywalow-Bartnicka P, Sikora E, **Skowronek K**, McKenna SL, Ghizzoni M, Dekker FJ, Piwocka K**.** Increased acetylation of lysine 317/320 of p53 caused by BCR-ABL protects from cytoplasmic translocation of p53 and mitochondria-dependent apoptosis in response to DNA damage. Apoptosis, 2012; 17(9):950-63
- Roszczenko P, Radomska KA, **Wywial E**, Collet JF, Jagusztyn-Krynicka EK. A novel insight into the oxidoreductase activity of Helicobacter pylori HP0231 protein. PLoS One, 2012; 7(10): e46563
- Al-Haggar M, Madej-Pilarczyk A, **Kozlowski L, Bujnicki JM**, Yahia S, Abdel-Hadi D, Shams A, Ahmad N, Hamed S, Puzianowska-Kuznicka M. A novel homozygous p.Arg527Leu LMNA mutation in two unrelated egyptian families causes overlapping mandibuloacral dysplasia and progeria syndrome. Eur J Hum Genet, 2012; 20(11):1134-40
- **Magnus M, Pawlowski M, Bujnicki JM**. MetaLocGramN: A meta-predictor of protein subcellular localization for Gram-negative bacteria. Biochim Biophys Acta, 2012; 1824(12):1425-33
- **Skowronek K, Boniecki MJ, Kluge B, Bujnicki JM**. Rational engineering of sequence specificity in R.MwoI restriction endonuclease. Nucleic Acids Res, 2012; 40(17):8579-92
- **Poleszak K, Kaminska KH, Dunin-Horkawicz S**, Lupas A, **Skowronek KJ, Bujnicki JM**. Delineation of structural domains and identification of functionally important residues in DNA repair enzyme Exonuclease VII. Nucleic Acids Res, 2012; 40(16):8163-74
- **Korneta I, Magnus M, Bujnicki JM**. Structural bioinformatics of the human spliceosomal proteome. Nucleic Acids Res, 2012; 40(15):7046-65
- Drozdz M, Piekarowicz A, **Bujnicki JM**, Radlinska M. Novel non-specific DNA adenine methyltransferases. Nucleic Acids Res, 2012; 40(5):2119-30
- **Sulej AA, Tuszynska I, Skowronek KJ,** Nowotny M, **Bujnicki JM.** Sequence-specific cleavage of the RNA strand in DNA-RNA hybrids by the fusion of ribonuclease H with a zinc finger. Nucleic Acids Res, 2012; 40(22):11563-70
- **Pawlowski M, Bujnicki JM**. The utility of comparative models and the local model quality for protein crystal structure determination by Molecular Replacement. BMC Bioinformatics, 2012; 13(1):289
- **Pietal MJ**, Szostak N, **Rother KM, Bujnicki JM**. RNAmap2D -- calculation, visualization and analysis of contact and distance maps for RNA and protein-RNA complex structures. BMC Bioinformatics, 2012; 13(1): 333
- **Siwek W,** Czapinska H, Bochtler M, **Bujnicki JM, Skowronek K**. Crystal structure and mechanism of action of the N6-methyladenine dependent type IIM restriction endonuclease. Nucleic Acids Res, 2012; 40(15):7563-72
- Samluk L, Czeredys M, **Skowronek K**, Nałęcz KA. Protein kinase C regulates amino acid transporter ATB. Biochem Biophys Res Commun, 2012; 422(1): 64-9
- Malenda A, Skrobanska A, Issat T, Winiarska M, Bil J, Oleszczak B, Sinski M, Firczuk M, **Bujnicki JM**, Chlebowska J, Staruch AD, Glodkowska-Mrowka E, Kunikowska J, Krolicki L, Szablewski L, Gaciong Z, Koziak K, Jakobisiak M, Golab J, Nowis DA. Statins Impair Glucose Uptake in Tumor Cells. Neoplasia, 2012; 14:311-23
- **Lach G**, DeKievet M, Jentschura UD. Einstein–Hopf drag, Doppler shift of thermal radiation and blackbody drag: Three perspectives on quantum friction. Cent Eur J Phys, 2012; 10(4):763-767
- Fislage M, Roovers M, **Tuszynska I, Bujnicki JM**, Droogmans L, Versées W. Crystal structures of the tRNA:m(2) G6 methyltransferase Trm14/TrmN from two domains of life. Nucleic Acids Res, 2012; 40(11):5149-61
- Cruz JA, Blanchet MF, **Boniecki M, Bujnicki JM**, Chen SJ, Cao S, Das R, Ding F, Dokholyan NV, Flores SC, Huang L, Lavender CA, Lisi V, Major F, **Mikolajczak K**, Patel DJ, **Philips A**, Puton T, Santalucia J, Sijenyi F, Hermann T, Rother K, Rother M, Serganov A, Skorupski M, **Soltysinski T**, Sripakdeevong P, **Tuszynska I**, Weeks KM, Waldsich C, Wildauer M, Leontis NB, Westhof E. RNA-Puzzles: A CASP-like evaluation of RNA three-dimensional structure prediction. RNA, 2012; 18:610-625
- **Chojnowski G, Bujnicki JM,** Bochtler M. RIBER/DIBER: a software suite for crystal content analysis in the studies of protein-nucleic acid complexes. Bioinformatics, 2012; 28(6):880-881
- Zylicz-Stachula A, Zolnierkiewicz O, Lubys A, Ramanauskaite D, Mitkaite G, **Bujnicki JM**, Skowron P. Related bifunctional restriction endonuclease-methyltransferase triplets: TspDTI, Tth111II/TthHB27I and TsoI with distinct specificities. BMC Mol Biol, 2012; 13(1):13
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- Nakagome S, Mano S, **Kozlowski L, Bujnicki JM**, Shibata H, Fukumaki Y, Kidd JR, Kidd KK, Kawamura S, Oota H. Crohn's Disease Risk Alleles on the NOD2 Locus Have Been Maintained by Natural Selection on Standing Variation. Mol Biol Evol, 2012; 29(6):1569-85
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- **Philips A, Milanowska K, Lach G, Boniecki M**, Rother K, **Bujnicki JM**. MetalionRNA: computational predictor of metal-binding sites in RNA structures. Bioinformatics, 2012; 28(2):198-205
- **Rother K, Rother M, Boniecki M, Puton T, Tomala K, Lukasz P, Bujnicki JM**. Template-based and template-free modeling of RNA 3D structure: inspirations from protein structure modeling In "RNA Structure Prediction and Modelling". Editors: Leontis NB, Westhof E, Springer, 2012, ISBN: 978-3-642-25739-1
- **Kozlowski L, Orlowski J, Bujnicki JM**. Structure prediction of alternatively spliced proteins In „Alternative pre-mRNA Splicing: Theory and Protocols: The Complete Guide for Biomedical Scientists". Editors: Stamm S, Smith C, Luhrmann R, Wiley-Blackwell, 2012, ISBN: 978-3-527-32606-8

Current Research

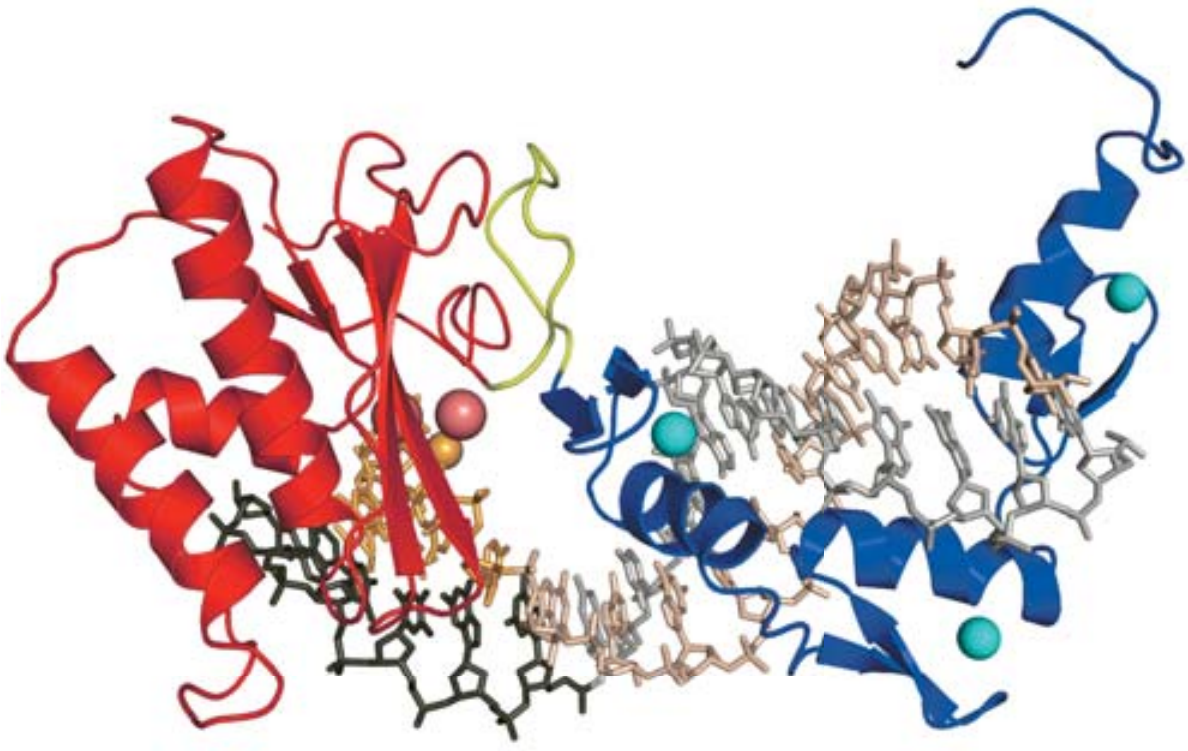
The Laboratory of Bioinformatics and Protein Engineering is involved in theoretical and experimental research on sequence-structure-function relationships in proteins and nucleic acids and macromolecular complexes. Theoretical research involves the development of computer software for the analysis of biological macromolecules. Currently, the focus is on the development of software for the structural prediction and modeling of RNA and RNA-protein complexes. Thus far, we have developed and made publicly available one of the first methods for the automated comparative modeling of RNA (ModeRNA; http://iimcb.genesilico.pl/moderna/), a method for the structure-based prediction of metal ion binding sites (MetalionRNA; http://metalionrna.genesilico.pl/), and statistical potentials for predicting the structure of RNA-protein complexes (DARS-RNP and QUASI-RNP; http://iimcb.genesilico.pl/RNP/).

Our suite of programs for protein structure prediction and analysis include the GeneSilico MetaServer for primary, secondary, and tertiary structure prediction (https://www.genesilico.pl/meta2/), a method for the quality assessment of protein models (MetaMQAP; https://genesilico.pl/toolkit/unimod?method=MetaMQAPII), and a method for the discrimination of models according to their agreement with experimental data (FILTREST3D; http://filtrest3d.genesilico.pl/). We also developed methods for the prediction of order/disorder in protein structures (http://iimcb.genesilico.pl/metadisorder/) and protein localization in Gram-negative bacterial cells (MetaLocGramN; http://genesilico.pl/MetaLocGramN/). We also developed a system of nucleic acid metabolism databases. Published elements of this system include MODOMICS (i.e., a database for the systems biology of RNA modification; http://modomics.genesilico.pl/), REPAIRtoire (i.e., a database for the systems biology of DNA repair; http://repairtoire.genesilico.pl/), and RNApathwaysDB (i.e., a database of pathways of RNA maturation and decay; http://genesilico.pl/rnapathwaysdb/).

Our experimental research is focused on the elucidation of sequence-structure-function relationships in proteins and nucleic acids using biophysics, biochemistry, molecular biology, and cell biology. Three principal types of analyses are performed by researchers in our wet lab:

- Experimental testing of functional predictions by gene cloning or nucleic acid synthesis, protein or RNA expression, purification, development of *in vitro* and *in vivo* functional assays, and biochemical and cellular characterization.
- Experimental testing of protein or RNA structural predictions by application of low-resolution structural probing methods, such as mutagenesis, chemical modification, crosslinking, mass spectrometry, and circular dichroism.
- Protein engineering to obtain enzymes with new, useful features, particularly altered substrate specificity (e.g., nucleases that recognize and cut new sequences in DNA or RNA).

Our theoretical and experimental research is tightly integrated, demonstrated by the publication of articles that comprise a combination of theoretical and experimental analyses (e.g., prediction and characterization of new enzymes). Protein engineering involves iterative protein structure model building, model-based experimental planning, a series of experimental analyses, and experiment-based improvement of the models and tools used for model building.



Recent highlights

Successful development of an enzyme that sequence-specifically cuts RNA in RNA/DNA hybrids: a case study

Ribonucleases (RNases) are valuable tools applied in the analysis of RNA sequences, structures, and function. Their substrate specificity is limited to the recognition of single bases or distinct secondary structures in the substrate. Currently, no RNases are available for the purely sequence-dependent fragmentation of RNA. Researchers from the Bujnicki laboratory, in collaboration with Dr. Nowotny (Head of Laboratory of Protein Structure at IIMCB), have developed a new enzyme that cleaves the RNA strand in DNA-RNA hybrids five nucleotides from a specific recognition sequence. The design involved a combination of computational structure prediction and experimental analyses. The engineered enzyme was a fusion of two functionally distinct domains: RNase HI that hydrolyzes RNA in DNA-RNA hybrids in a processive and sequence-independent manner and a zinc finger that recognizes a sequence in DNA-RNA hybrids.

Methods for engineering zinc finger domains with new sequence specificities are readily available, making the acquisition of a library of RNases that recognize and cleave various sequences feasible, much like the commercially available assortment of restriction enzymes. Zinc finger-RNase HI fusions, in addition to *in vivo* applications, may potentially be used *in vivo* for targeted RNA degradation. The results of this research are the subject of a patent application and have been published (Sulej et al., *Nucleic Acids Res*, 2012, 40:11563-115670).

Computational structural analysis of mRNA-splicing machinery in human cells: a case study

The spliceosome is one of the largest molecular machines known. It excises introns from eukaryotic pre-mRNAs. In human cells, it comprises five RNAs, over 100 "core" proteins, and more than 100 additional associated proteins. The details of the spliceosome mechanism of action are unclear because only a small fraction of spliceosomal proteins have been characterized structurally in high resolution.

Researchers from the Bujnicki laboratory have performed a comprehensive analysis of the human spliceosomal proteome. They discovered that almost half of the combined sequence of proteins that are abundant in the spliceosome are predicted to be intrinsically disordered, at least when the individual proteins are considered in isolation. They also correlated the type and abundance of disordered proteins with different protein functions. For regions without an experimental structure in the ordered part of the spliceosomal proteome, they predicted and modeled three-dimensional structures. They also developed a database of structural models for the entire spliceosomal proteome, called SpliProt3D. The results of this work enable multiscale modeling of the structure and dynamics of the entire spliceosome and its subcomplexes and will guide further research toward understanding the molecular mechanism of mRNA splicing. The results of this research have been published (Korneta et al., *Nucleic Acids Res*, 2012, 40:7046-7065; Korneta and Bujnicki, *PLoS Comput Biol*, 2012, 8:e1002641).

Structures of C5 methyltransferases in productive complexes with target DNA. Only the structure of M.MpeI with DNA is our work, the other two structures were drawn according to coordinates from other laboratories.

Laboratory of **Structural Biology**

Lab leader: **Matthias Bochtler**, PhD, Professor

Postdoctoral Fellows:
Honorata Czapińska, PhD
Agnieszka Kolano, PhD (since January 2013)
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Katarzyna Misztal, PhD (since June 2012)
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Marta Szychowska, Eng
Patrycja Wawrzyniecka, BSc (IBB PAN)

Technician:
Agnieszka Olszewska (part-time)

Grant Administrator and Administrative assistant:
Izabela Zacharek, MSc (part-time)

Head of Laboratory of Structural Biology

Matthias Bochtler, PhD, Professor

Degrees

2009	Professor of Biological Sciences, nomination by the President of the Republic of Poland
2006	DSc Habil, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
1999	PhD in Biochemistry, Technical University of Munich, Germany
1995	MSc in Experimental Physics, Munich University, Germany

Research Training

1996-1999	Research Assistant, Max Planck Institute of Biochemistry, Martinsried, Germany
1995-1996	Internship, Medical Microbiology, University of Regensburg, Germany
1992-1993	Guest Student, Cambridge University, United Kingdom
1990-1992	Studies in physics, Munich University, Germany

Professional Employment

2011-Present	Head, Structural Biology Laboratory, International Institute of Molecular and Cell Biology and Institute of Biochemistry and Biophysics, Warsaw, Poland
2007-2011	Part-time Director of Structural Biology, Cardiff University, United Kingdom
2001-2010	Head, Joint MPG-PAN Junior Research Group, IIMCB, Warsaw, Poland
2000	Patent training, Weickmann & Weickmann
1999-2000	Postdoctoral Fellow, Max Planck Institute of Biochemistry, Martinsried, Germany

Honors, Prizes, Awards

2011	Full Professor, Institute of Biochemistry and Biophysics PAN, Warsaw
2005	Pieńkowski Award
2004	EMBO/HHMI Young Investigator Award
2000	Crystal Award, Germany
1998	Crystal Award, Germany
1990-1992	Scholarship from Deutsche Studienstiftung and Bavarian State

Selected publications

Protein-nucleic acid interactions

- **Wojciechowski M, Czapinska H, Bochtler M**. CpG Underrepresentation and the Bacterial CpG Specific DNA Methyltransferase M.MpeI. Proc Natl Acad Sci USA, 2013; 110(1):105-110
- **Bochtler M**. Structural basis of the TAL effector-DNA interaction. Biol Chem, 2012; 393(10):1055-66
- **Siwek W, Czapinska H, Bochtler M, Bujnicki JM, Skowronek K**. Crystal structure and mechanism of action of the N6-methyladenine dependent type IIM restriction endonuclease. Nucleic Acids Res, 2012; 40(15):7563-72
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- **Sokolowska M, Kaus-Drobek M, Czapinska H**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Monomeric restriction endonuclease BcnI in the apo form and in an asymmetric complex with target DNA. J Mol Biol, 2007; 369:722-34
- **Kaus-Drobek M, Czapinska H, Sokolowska M**, Tamulaitis G, **Szczepanowski RH**, Urbanke C, Siksnys V, **Bochtler M**. Restriction endonuclease MvaI is a monomer that recognizes its target sequence asymmetrically. Nucleic Acids Res, 2007; 35:2035-46
- **Bochtler M, Szczepanowski RH**, Tamulaitis G, Grazulis S, **Czapinska H**, Manakova E, Siksnys V. Nucleotide flips determine the specificity of the Ecl18kI restriction endonuclease. EMBO J, 2006; 25:2219-29
- Grazulis S, Manakova E, Rössle M, **Bochtler M**, Tamulaitiene G, Huber R, Siksnys V. Structure of the metal-independent restriction enzyme BfiI reveals fusion of a specific DNAbinding domain with a nonspecific nuclease. Proc Natl Acad Sci USA, 2005; 102:15797-802

Other

- **Sabala I**, Jonsson IM, Tarkowski A, **Bochtler M**. Anti-staphylococcal activities of lysostaphin and LytM catalytic domain. BMC Microbiol, 2012; 12:97
- **Chojnowski G, Bochtler M**. DIBER: protein, DNA or both? Acta Crystallogr D, 2010; 66:643-653
- Gentsch M, **Kaczmarczyk A**, van Leeuwen K, de Boer M, **Kaus-Drobek M**, Dagher MC, Kaiser P, Arkwright PD, Gahr M, Rösen-Wolff A, **Bochtler M**, Secord E, Britto-Williams P, Saifi GM, Maddalena A, Dbaibo G, Bustamante J, Casanova JL, Roos D, Roesler J. Alu-repeat-induced deletions within the NCF2 gene causing p67-phoxdeficient chronic granulomatous disease (CGD). Hum Mutat, 2010; 31:151-158
- **Chojnowski G**, Breer K, Narczyk M, Wielgus-Kutrowska B, **Czapinska H**, Hashimoto M, Hikishima S, Yokomatsu T, **Bochtler M**, Girstun A, Staron K, Bzowska A. 1.45 A resolution crystal structure of recombinant PNP in complex with a pM multisubstrate analogue inhibitor bearing one feature of the postulated transition state. Biochem Biophys Res Commun, 2010; 391:703-708
- **Piano D, El Alaoui S, Korza HJ, Filipek R, Sabala I, Haniewicz P**, Buechel C, De Sanctis D, **Bochtler M**. Crystallization of the photosystem II core complex and its chlorophyll binding subunit CP43 from transplastomic plants of Nicotiana tabacum. Photosyn. Res, 2010; 106:221-226

Scientific Report

Our group currently works on sequence- and modification-specific protein-nucleic acid interactions. With an FNP Team grant to support our work, the focus is now almost exclusively on DNA methylation and hydroxymethylation. These modifications are present in prokaryotes and eukaryotes, but they have very different roles in these organisms. Our group seeks to exploit the prokaryotic biology of DNA methylation and hydroxymethylation to develop tools for the study of these modifications in eukaryotes, particularly zebrafish and mice. In 2012, our efforts were concentrated on one story that emphasizes the deep evolutionary roots of DNA methylation.

DNA cytosine methylation in many eukaryotic species is predominantly found in the context of the CpG dinucleotide. It is thought to be essential in mammals and many other model animals, with the notable exception of the fruit fly *Drosophila melanogaster*. However, cytosine methylation comes at a price. Genome-wide studies have consistently shown that CpG methylation in eukaryotes is associated with CpG depletion. As a result, the CpG dinucleotide is found several-fold less frequently in nuclear DNA of higher mammals, including humans, than one might expect based on the GC content of the DNA. The reasons for the link between CpG methylation and depletion are both chemical and biological. At the chemical level, cytosine methylation promotes deamination and leads to thymines. More importantly, at the biological level, methylcytosines converted to thymines are difficult to identify as a damage product and difficult to repair to cytosines through DNA repair pathways. Both the chemical and biological arguments for the link between CpG methylation and depletion are fairly fundamental and should apply to all kingdoms of life. Hence, one can ask the question, "Is it possible to discover novel prokaryotic CpG methyltransferases (like the previously found CpG specific M.SssI) by searching bacterial genomes for CpG depletion?" We have performed exactly this and scanned all fully sequenced bacterial genomes in the NCBI sequence collection for CpG underrepresentation. We found several drastically (i.e., approximately 10-fold) CpG-depleted bacterial species. Although we found differences in the statistical signatures of CpG depletion when compared with eukaryotes (e.g., in the comparison of coding and non-coding regions), we followed this observation by analyzing bacteria with drastic CpG depletion for CpG methylation. In the case of *Mycoplasma penetrans*, a genome-wide study of cytosine methylation using bisulfite sequencing identified global CpG methylation and several other universally methylated sequences. To identify the *M. penetrans* CpG methyltransferase, we picked a candidate protein on the basis of remote amino acid sequence similarity to M.SssI. Using bisulfite sequencing and other CpG methylation assays (i.e., HpaII/MspI digestion), we demonstrated *in vitro* that our candidate enzyme was indeed a CpG-specific DNA methyltransferase and hence named it M.MpeI in accordance with nomenclature guidelines.

How does M.MpeI recognize its CpG target sequence with extraordinary specificity? To answer this question, we crystallized M.MpeI with target DNA and solved the structure. Unsurprisingly, we found typical features of CpG methyltransferase DNA complexes, such as flipping of the substrate cytosine and the proximity of the co-factor to the substrate base for direct transfer of a methyl group. Very interestingly, the DNA structure was perturbed not only in the substrate strand but also in the complementary strand. In this strand, we detected intercalation of a phenylalanine residue between the C and G nucleotides of the CpG site. The 5'-pyrimidine purine-3' steps are thought to be easier to unstack than other dinucleotide steps. Hence, intercalation might contribute to CpG readout. This concept is supported by the recent structure of the eukaryotic DNA

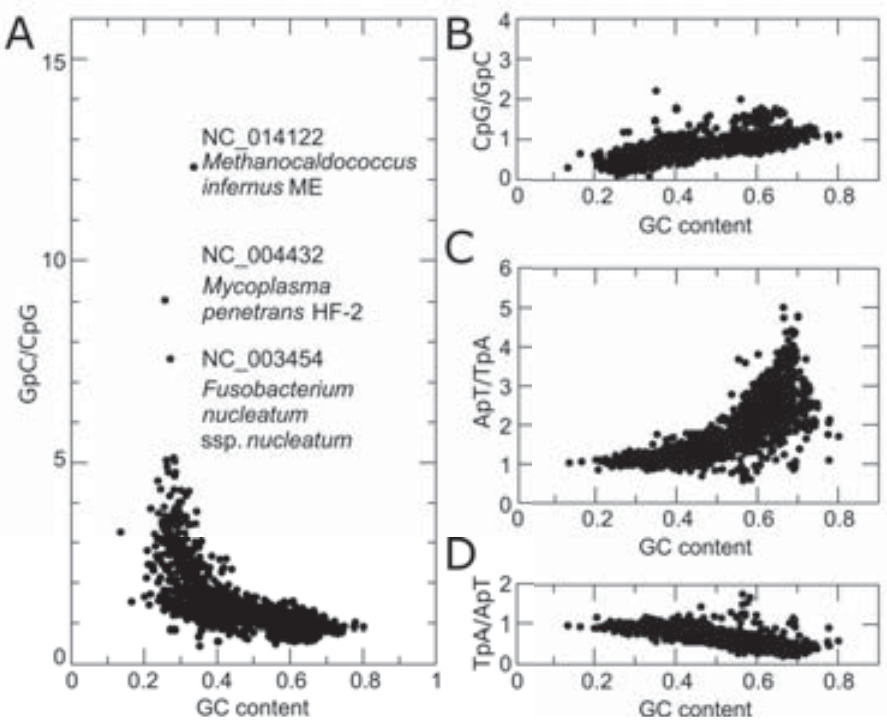


Fig. 1. (A) Initial screen for CpG depletion in bacterial genomes. The GpC/CpG dinucleotide ratio was used as a "proxy" for CpG depletion in order to normalize for the GC content. Every dot in the diagram represents one bacterial genome. (B-D) The same for related dinucleotide ratios as a control.

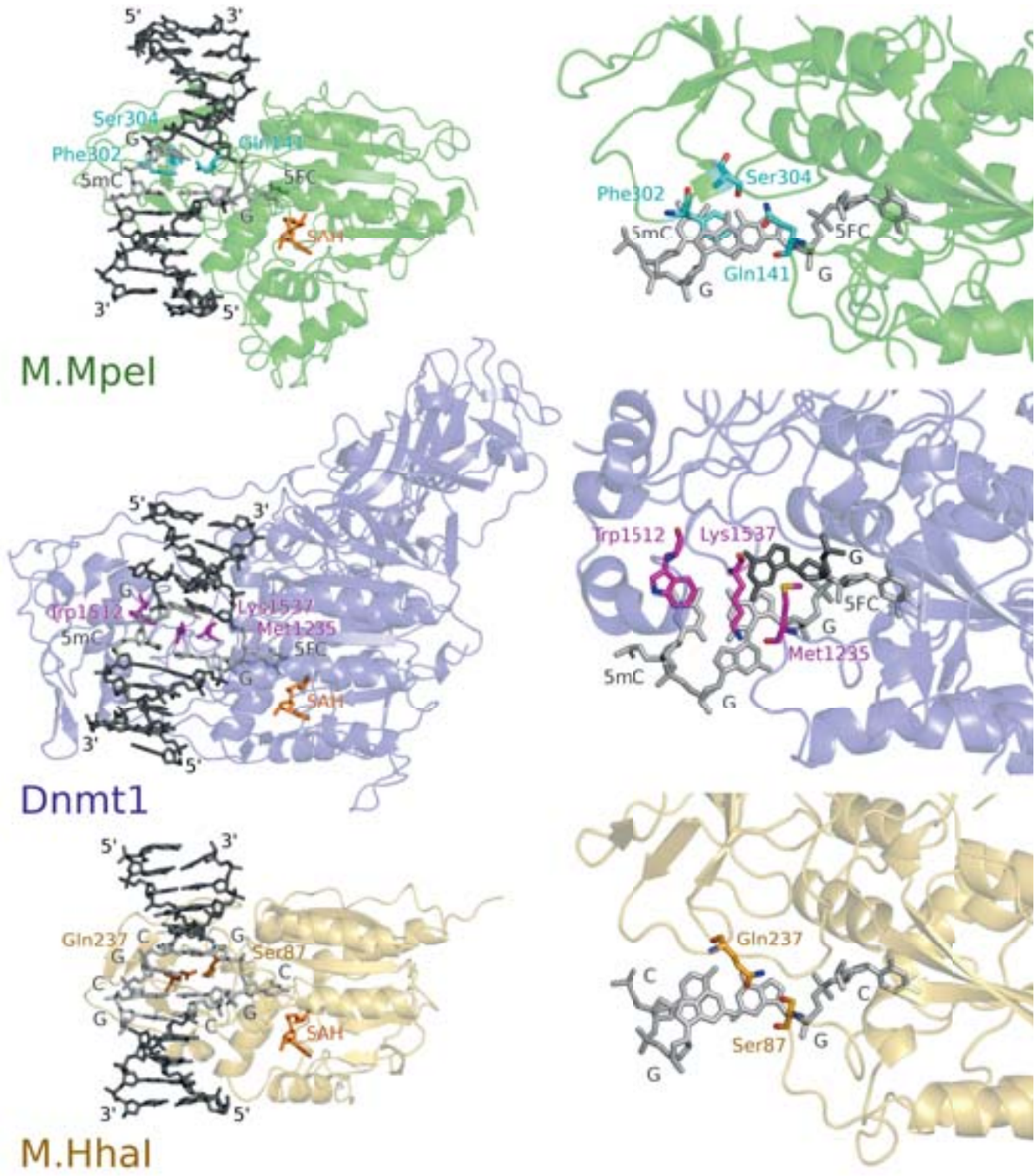
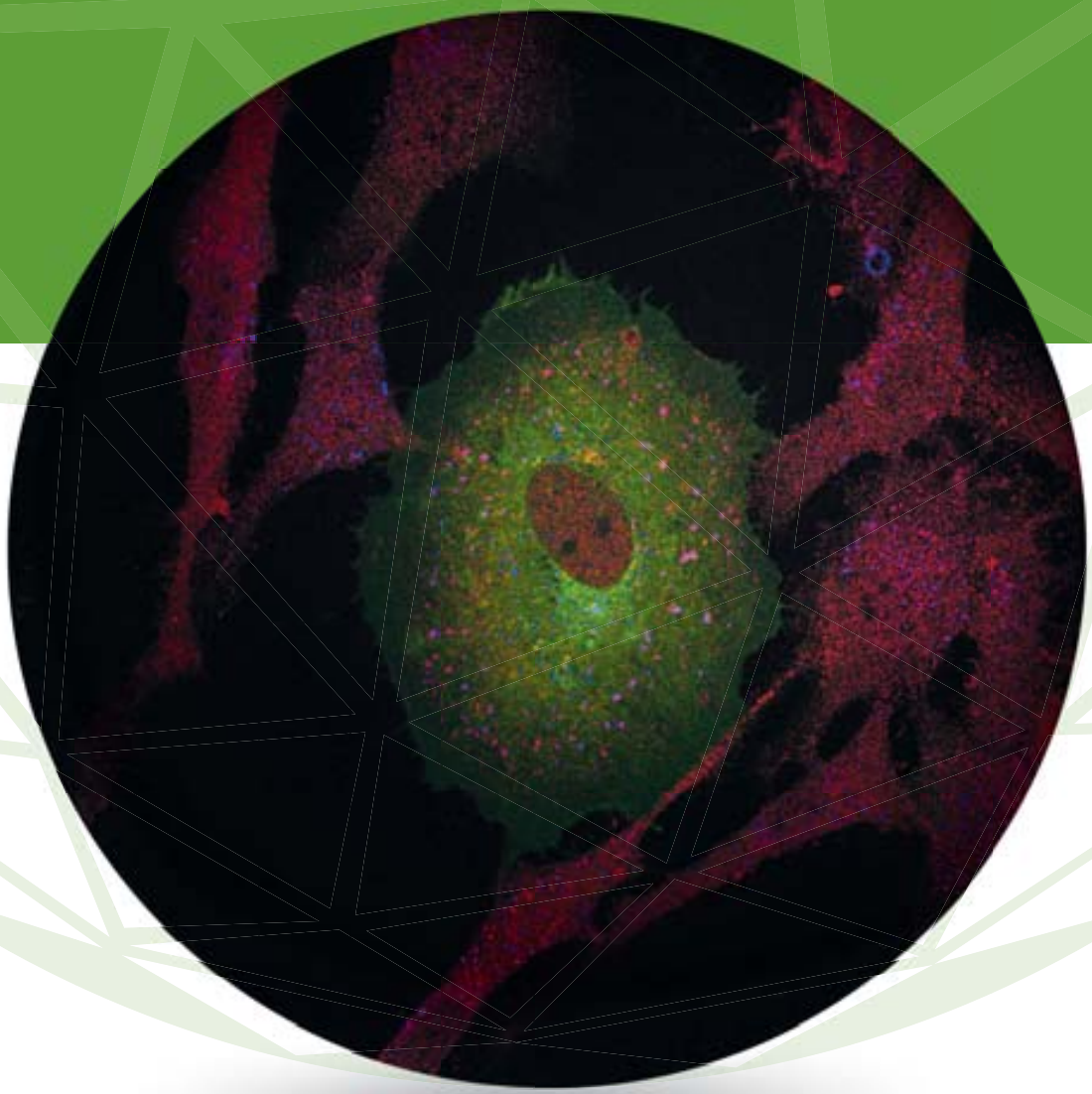


Fig. 2. Structures of C5 methyltransferases in productive complexes with target DNA. Only the structure of M.MpeI with DNA is our work, the other two structures were drawn according to coordinates from other laboratories.

maintenance methyltransferase Dnmt1 in complex with target DNA, which also shows unstacking of the CpG step.

If CpG methylation damages genomes, then what is the benefit for bacteria to retain a CpG-specific DNA methyltransferase? We are presently unable to answer this question, but several possibilities exist. The methyltransferase might be part of a CpG-specific restriction modification system. Alternatively and somewhat improbably in light of our genome-wide methylation data, it might play a role as an epigenetic regulator. Finally, bacterial CpG methylation might involve host pathogen interactions. Although the claim is still debated, most authors now agree that CpG-unmethylated DNA is far more immunogenic than CpG-methylated DNA. Hence, CpG methylation might help bacteria dodge the host immune system. If so, then our findings could also have medical applications because at least some of the CpG-specific DNA methyltransferases are found in human pathogens.



Human fibroblasts that overexpress GFP-Cdc42-L61 (green) stained for PDGFRβ (red) and EEA1 (blue) (Author: Kamil Jastrzębski).

Laboratory of **Cell Biology**

Lab leader: **Marta Miączyńska**, PhD, DSc Habil

Postdoctoral Fellows:
Magdalena Banach-Orłowska, PhD
Anna Bartosik, PhD (since Feb. 2013)
Iwona Pilecka, PhD (until March 2012)
Beata Pyrzyńska, PhD
Ewelina Szymańska, PhD

Junior Researchers:
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Agnieszka Mamińska, MSc
Łukasz Sadowski, MSc
Sam D. Stephen, MSc
Anna Toruń, MSc

FishMed Technical Assistant:
Lidia Wolińska, MSc (since Jan. 2013)

Trainees:
Agnieszka Skowronek, MSc (March-December 2012)

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Technician:
Monika Matuszczyk (on leave since March 2012)
Alina Zielińska (since April 2012)

Head of Laboratory of Cell Biology

Marta Miączyńska, PhD, DSc Habil

Degrees

2008	DSc Habil in Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1997	PhD in Genetics, University of Vienna, Austria
1993	MSc in Molecular Biology, Jagiellonian University, Cracow, Poland
1991	BSc in Biological Sciences, University of Wolverhampton, UK

Research Training

2001-2005	Senior Postdoctoral Fellow, Max Planck Institute for Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany
1997-2000	Postdoctoral training, European Molecular Biology Laboratory, Heidelberg, Germany
1993-1996	PhD studies, Institute of Microbiology and Genetics, University of Vienna, Austria
1990-1991	Exchange Student, University of Wolverhampton, UK

Fellowships and Awards

2007	Habilitation Fellowship of L'Oreal Poland for Women in Science
2005	International Research Scholar, Howard Hughes Medical Institute, USA (2006-2010)
2005	International Senior Research Fellowship, Wellcome Trust, UK (2006-2011)
2005	Partner Group grant, Max Planck Society, Germany (2006-2010)
2001-2004	Postdoctoral Fellowship, Max Planck Society, Germany
1999-2000	Long-Term Postdoctoral Fellowship, Human Frontier Science Program Organization (HFSPO)
1998-1999	Erwin Schrödinger Postdoctoral Fellowship, Austrian Science Fund (FWF)
1993-1996	Bertha von Suttner PhD Scholarship, Austrian Ministry of Science
1990-1991	Studentship, European Community Tempus Scheme

Selected publications

- **Pyrzynska B, Banach-Orlowska M, Teperek-Tkacz M**, Miekus K, Drabik G, Majka M, **Miaczynska M**. Multifunctional protein APPL2 contributes to survival of human glioma cells. Mol Oncol, 2013; 7:67-84
- Winiarska M, Nowis D, Bil J, Glodkowska-Mrowka E, Muchowicz A, Wanczyk M, Bojarczuk K, Dwojak M, Firczuk M, Wilczek E, Wachowska M, Roszczenko K, **Miaczynska M**, Chlebowska J, Basak GW, Golab J. Prenyltransferases Regulate CD20 Protein Levels and Influence Anti-CD20 Monoclonal Antibody-mediated Activation of Complement-dependent Cytotoxicity. J Biol Chem, 2012; 287:31983-93
- Zerrouqi A, **Pyrzynska B**, Febbraio M, Brat DJ, Van Meir EG. p14ARF inhibits human glioblastoma-induced angiogenesis by upregulating the expression of TIMP3. J Clin Invest, 2012; 122:1283-95
- **Hupalowska A, Pyrzynska B, Miaczynska M**. APPL1 regulates basal NF-κB activity by stabilizing NIK. J Cell Sci, 2012; 125: 4090-102
- **Hupalowska A**, **Miaczynska M**. The new faces of endocytosis in signaling. (Review) Traffic, 2012; 13:9-18
- **Urbanska A**, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Biochemical Characterization of APPL Endosomes: The Role of Annexin A2 in APPL Membrane Recruitment. Traffic, 2011; 12:1227-41
- **Pilecka I**, **Sadowski L**, Kalaidzidis Y, **Miaczynska M**. Recruitment of APPL1 to ubiquitin-rich aggresomes in response to proteasomal impairment. Exp Cell Res, 2011; 317:1093-107
- **Miaczynska M**, Bar-Sagi D; Signaling endosomes: seeing is believing. (Review) Curr Opin Cell Biol, 2010; 22:535-540
- **Banach-Orlowska M, Pilecka I, Torun A, Pyrzynska B, Miaczynska M**. Functional characterization of the interactions between endosomal adaptor protein APPL1 and the NuRD co-repressor complex. Biochem J, 2009; 423:389–400
- **Pyrzynska B, Pilecka I, Miaczynska M**. Endocytic proteins in the regulation of nuclear signaling, transcription and tumorigenesis. (Review) Mol Oncol, 2009; 3: 321-338
- **Rashid S, Pilecka I, Torun A, Olchowik M, Bielinska B, Miaczynska M**. Endosomal Adaptor Proteins APPL1 and APPL2 Are Novel Activators of beta-Catenin/TCF-mediated Transcription. J Biol Chem, 2009; 284:18115-28
- **Sadowski L, Pilecka I, Miaczynska M**. Signaling from endosomes: Location makes a difference. (Review) Exp Cell Res, 2009; 315:1601-09
- *Ohya T, **Miaczynska M**, Coskun U, Lommer B, Runge A, Drechsel D, Kalaidzidis Y, Zerial M. Reconstitution of Rab and SNARE-dependent membrane fusion by synthetic endosomes. Nature, 2009; 459:1091-97
- **Miaczynska M**, Stenmark H. Mechanisms and functions of endocytosis. J Cell Biol, 2008; 80:7-11
- **Pilecka I, Banach-Orlowska M, Miaczynska M**. Nuclear functions of endocytic proteins. Eur J Cell Biol, 2007; 86:533-547
- *Mace G, **Miaczynska M**, Zerial M, Nebreda AR. Phosphorylation of EEA1 by p38 MAP kinase regulates μ opioid receptor endocytosis. EMBO J, 2005; 24:3235-46
- ***Miaczynska M**, Pelkmans L, Zerial M. Not just a sink: endosomes in control of signal transduction. (Review) Curr Opin Cell Biol, 2004; 16:400-406
- ***Miaczynska M**, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M. APPL proteins link Rab5 to signal transduction via an endosomal compartment. Cell, 2004; 116:445-56

* no IIMCB affiliation

Description of Current Research

Our major research interest concerns the mutual relationship between the processes of intracellular signal transduction and membrane trafficking. We study the molecular mechanisms by which endocytic transport regulates intracellular signal transmission and affects final signaling output. The specific projects developed by our group follow two general lines of investigation, with the aim of clarifying the following:

I. Role of endosomal compartments in the trafficking and signaling of growth factors.

II. Involvement of endocytic proteins in the regulation of intracellular signaling and gene expression in the nucleus.

The intracellular compartmentalization of signal transduction processes may play an important role in modulating the overall cellular response. Endocytosis was first viewed simply as a mechanism of signal termination by the downregulation and degradation of surface receptors. However, more recent data strongly argue that endosomal compartments and their resident proteins play an important role in transmitting intracellular signals by transporting ligand-receptor complexes and affecting their activity inside the cell (Hupalowska and Miaczynska, *Traffic*, 2012; Miaczynska and Bar-Sagi, *Curr Opin Cell Biol*, 2010). The proposal that endosomes serve as signaling compartments, which was initially postulated in the mid-1990s, has gained increasing experimental support in the past few years (Sadowski et al., *Exp Cell Res*, 2009).

Moreover, the relaying of signals from the plasma membrane via endosomes to the nucleus requires signal mediators to be transported between different cellular locations. Intriguingly, a growing number of clathrin adaptors and endosomal

proteins are reported to undergo nucleocytoplasmic shuttling. Endocytic proteins can interact with nuclear molecules involved in transcription or chromatin remodeling, changing their localization or activity, and thus may directly modulate the levels or specificity of gene transcription. Certain endocytic proteins translocate to the nucleus in response to extracellular signals to exert a specific biological effect, thus serving as a vehicle for molecular communication between intracellular organelles. In most other cases, however, unclear is the extent to which endocytic and nuclear functions are related or represent disparate tasks (so-called moonlighting; Pilecka et al., *Eur J Cell Biol*, 2007). Importantly, some such dual-function endocytic and nuclear proteins affect cell proliferation or act as tumor suppressors, or their expression is changed in human cancers (Pyrzynska et al., *Mol Oncol*, 2009).

Our direct links to both lines of research include previous studies of adaptor proteins APPL1 and APPL2. These homologous proteins are localized to a particular subpopulation of endosomes but can also act as signal transducers capable of nuclear translocation. As such, they provide examples of both the involvement of endosomes in signaling and the activity of endocytic proteins in the nucleus (Miaczynska et al., *Cell*, 2004). Our initial research efforts concentrated on APPL1 and APPL2 as exemplary proteins involved in endocytic trafficking and nuclear signaling, whereas more recently we have been extending our studies to exploring other dual-function endocytic proteins.

In 2012, we completed two projects, described below, in which we uncovered new functions of APPL proteins in intracellular signaling and transcriptional regulation.

Novel role of APPL1 in the regulation of NF-κB signaling (Hupalowska et al., *J Cell Sci*, 2012)

Accumulating evidence from several laboratories, including ours, indicated that the APPL1 protein actively participates in different signaling pathways. We uncovered a new function of APPL1 as a positive regulator of nuclear factor-kB (NF-κB) signaling in the absence of cytokine stimulation. Transcription factors of the NF-κB family are ubiquitously expressed in all cells. They function primarily in controlling immune and inflammatory responses, but they also regulate various other physiological processes, such as cell growth, differentiation, apoptosis, cell adhesion, and oxidative stress responses.

The starting point of the project was demonstrating a direct interaction between APPL1 and TRAF2, an adaptor protein known to activate the canonical NF-κB signaling. Although TRAF2 was not detectable on APPL endosomes, the endosomal recruitment of APPL1 was required for its function in the NF-κB pathway. We demonstrated that APPL1 synergized with TRAF2 in the induction of NF-κB activation upstream of the IkB kinase (IKK) complex. Consequently, APPL1 appeared to regulate the proper spatial distribution of RelA/p65, the canonical NF-κB transcription factor, in the absence of cytokine stimulation. Overexpression of APPL1 increased and its depletion decreased the nuclear accumulation of RelA/p65.

To obtain further molecular insights into the mechanisms of action of APPL1, we analyzed the patterns of gene transcription upon APPL1 overproduction and depletion. In both cases, we found altered expression of a specific subset of NF-κB target genes, including those involved in noncanonical signaling. In agreement with this observation, APPL1 overproduction

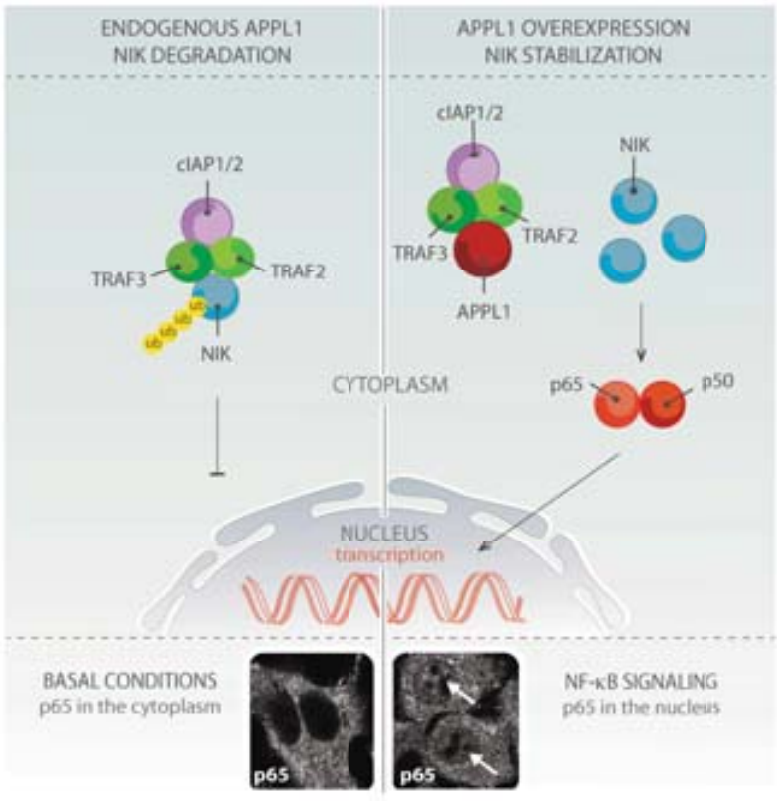


Fig. 1. Model of APPL1 function in NF-κB signaling.
Under basal conditions (left), NIK, a master kinase of noncanonical NF-κB signaling, is constitutively ubiquitinated by the degradative complex that consists of TRAF2, TRAF3, and cIAP1/2 proteins. The resulting destruction of NIK prevents transcriptional activation. Upon APPL1 overexpression (right), high levels of APPL1 that bind to TRAF2 reduce the association between NIK and its degradative complex, resulting in the stabilization of NIK. The increased accumulation of NIK activates the canonical branch of the NF-κB pathway, leading to nuclear translocation of the p65/p50 NF-κB dimer and transcriptional activation. The microscopic images show p65 transcription factor staining in the cytoplasm under basal conditions (left) and its increased nuclear accumulation upon APPL1 overexpression (right, marked by arrows), leading to enhanced NF-κB signaling (Author: Anna Hupałowska).

enhanced noncanonical NF-κB activation, reflected by increased NF-κB2/p100 processing to p52. We showed that this effect was attributable to the stabilization of NF-kB-inducing kinase (NIK), a key upstream kinase of the noncanonical pathway. Because of its ability to bind TRAF2, APPL1 overproduction reduced the association between NIK and the degradative complex that contains TRAF2, TRAF3, and cIAP1, thus causing the stabilization and cellular accumulation of NIK. In turn, high levels of NIK triggered the nuclear translocation of RelA/p65.

Based on these data, we proposed that APPL1 regulates NF-κB activity by modulating the stability of NIK in the noncanonical branch of the pathway (Fig. 1). However, NIK accumulation also leads to the activation of the canonical branch and eventually nuclear accumulation of RelA/p65. Thus, our study indicates that APPL1 is a novel link between the canonical and noncanonical machineries of NF-κB activation.

More broadly, our results indicate that endocytic proteins can regulate NF-κB signaling. Some trafficking proteins, such as Tollip, Tom1, and β-arrestins, were previously reported to inhibit the NF-κB pathway, but little was known about the involvement of endocytic proteins in the activation of the NF-κB pathway, as we demonstrated for APPL1. Our study also has more general mechanistic implications. We found mechanisms that link the canonical and noncanonical NF-κB signaling pathways in non-immune cells in the absence of cytokine stimulation (Fig. 1).

APPL2 contributes to the survival of human glioma cells by counteracting the induction of apoptosis (Pyrzynska et al., *Mol Oncol*, 2013)

We found that APPL2 protein levels are increased in approximately 40% of human samples of glioblastoma multiforme, the most common and aggressive cancer of the central nervous system. Using cultured cells, we showed that manipulating the levels of APPL2 changed the growth properties of glioma cells. In short-term experiments, the silencing of APPL2 expression by small-interfering RNA (siRNA) impaired cell survival, particularly under conditions of reduced growth factor availability, and enhanced apoptosis. In long-term experiments, APPL2 depletion by short-hairpin RNA (shRNA) inhibited the anchorage-independent growth of glioma cells in soft agar and their ability to grow as xenografts *in vivo*. In agreement with these findings, APPL2 overexpression exerted cytoprotective effects in glioma cells treated with apoptosis inducers.

At the molecular level, we excluded the possibility that the negative effect of APPL2 knockdown on cell survival was attributable to alterations in AKT or glycogen synthase kinase 3b (GSK3β) activity, which were reported to be modulated by APPL proteins. Instead, we attributed the APPL2 depletion-induced reduction of cell survival to changes in gene expression, particularly the upregulation of apoptosis-related genes, such as *UNC5B* (a pro-apoptotic dependence receptor) and *HRK* (harakiri, an activator of apoptosis that antagonizes the anti-apoptotic function of Bcl2). Their crucial role in regulating glioma cell viability was supported by our findings that the loss of cell survival upon APPL2 knockdown could be rescued by either simultaneous silencing HRK or excess netrin-1, the pro-survival ligand of UNC5B. APPL2 overexpression reduced HRK expression and caspase activation in cells treated with apoptosis inducers, resulting in enhanced cell viability. We further showed that this pro-survival activity of APPL2 occurred independently of its endosomal localization. We concluded that the increased levels of APPL2 in glioblastoma may confer a growth advantage by maintaining low expression levels of the genes responsible for the induction of cell death (Fig. 2).

To our knowledge, this is the first demonstration of the role of APPL2 in tumorigenesis. Accumulating evidence suggests that the aberrant endocytosis of signaling molecules may contribute to the development of various malignancies. Moreover, endocytic proteins also participate in cellular signaling and transcriptional regulation. Supporting this notion, our data demonstrated that modulating APPL2 levels affected the patterns of gene transcription, thus regulating the physiological responses of the cell, including survival and apoptosis, that in turn contributed to oncogenic transformation.

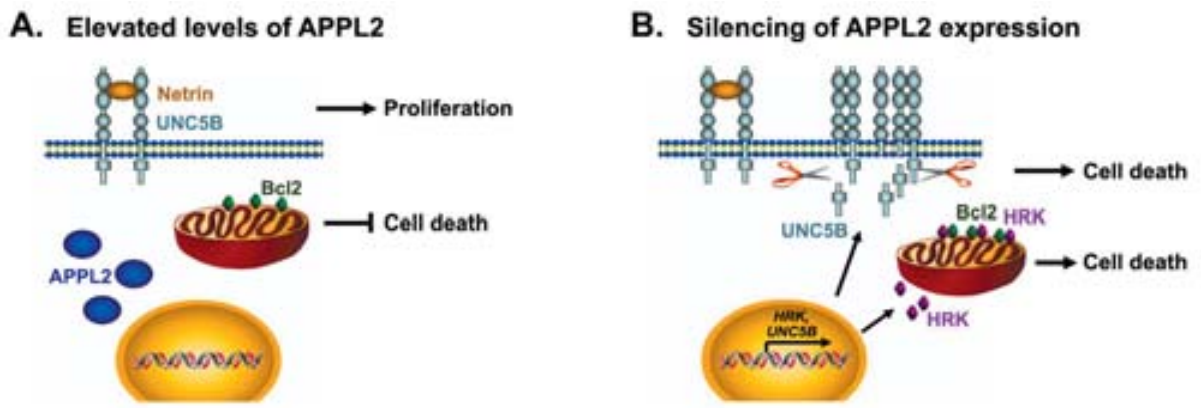
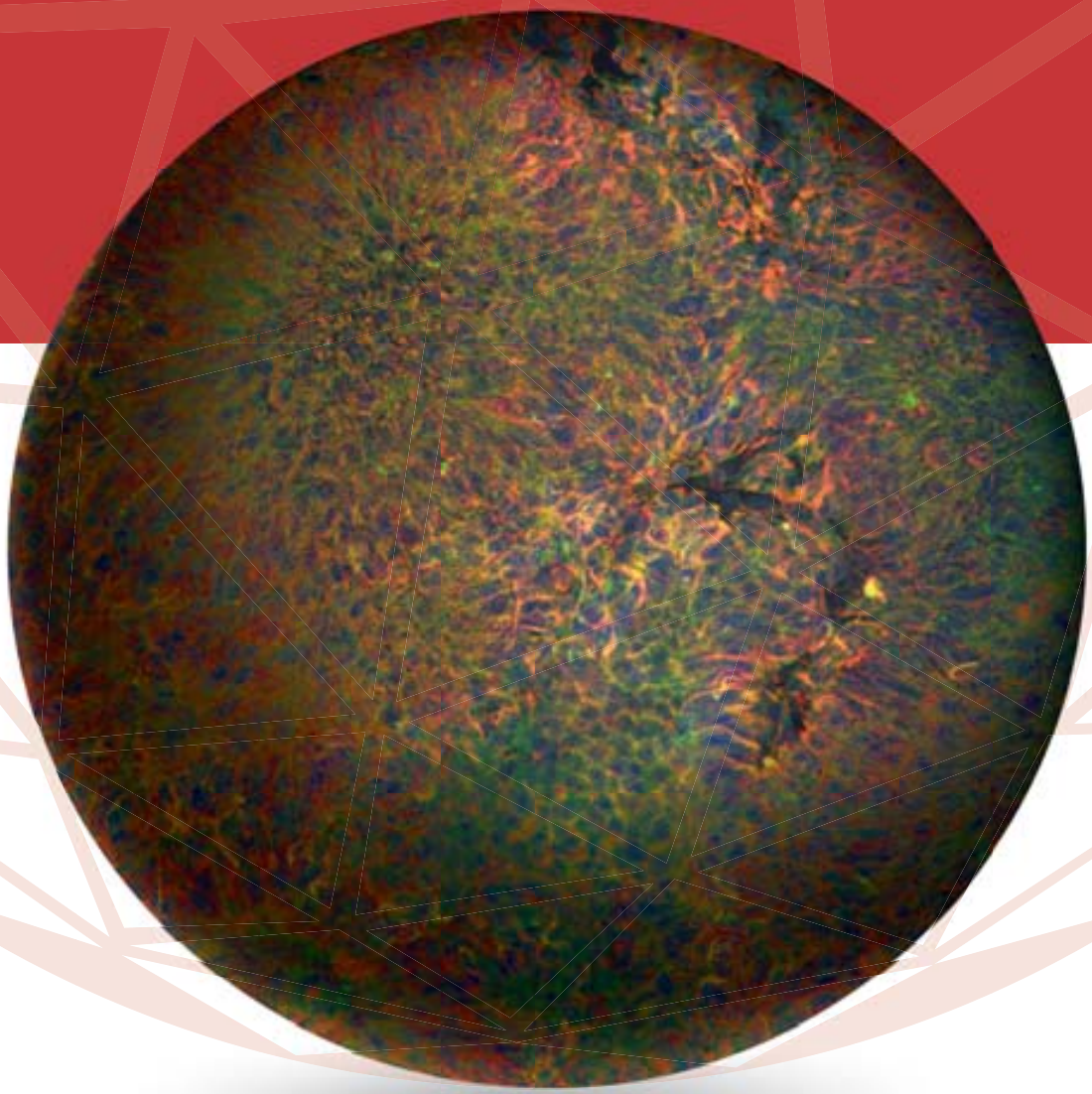


Fig. 2. Postulated mechanism of pro-survival activity of APPL2 in glioma cells.
(A) Glioma cells exhibit elevated levels of APPL2 protein. They express the transmembrane dependence receptor UNC5B and its secreted ligand netrin. Upon netrin binding, the UNC5B receptor transmits signals toward proliferation. In parallel, cell death is blocked by the anti-apoptotic action of the mitochondrial protein Bcl2, resulting in enhanced cell survival.
(B) The decrease in APPL2 level (achieved experimentally by siRNA-mediated silencing) stimulates the expression of the *HRK* and *UNC5B* genes. Excess UNC5B beyond the level of its ligand results in the presence of unbound receptors that undergo intracellular cleavage, accompanied by activation of caspases and induction of apoptosis. In parallel, the pro-apoptotic protein HRK binds to and antagonizes the action of Bcl2, resulting in the activation of caspases, followed by cell death (Author: Beata Pyrzyńska).



Induced pluripotent stem cell – derived neurons are used in the Laboratory of Molecular and Cellular Neurobiology to model human diseases. The microphotograph shows confocal image of human iPSC-derived neuronal rosettes stained for neuroprecursors markers – vimentin (green), nestin (red) and nuclear dye (blue). Photo by Ewa Liszewska.

Laboratory of **Molecular and Cellular Neurobiology**

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Ewa Liszewska, PhD
Matylda Macias, PhD
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Małgorzata Urbańska, MSc
Anna Bajur
Adrianna Łach
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FishMed Technical Assistant
Lidia Wolińska, MSc (since Jan. 2013)

Technicians:
Monika Matuszczyk
Marcelina Pieprzyk, MSc
Alina Zielińska

Head of Laboratory of Molecular and Cellular Neurobiology

Jacek Jaworski, PhD, DSc Habil

Degrees

2010	DSc Habil in Molecular Biology, Warsaw University, Poland
2001	PhD in Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1996	MSc in Biology, Department of Genetics, Warsaw University, Poland

Research Training

2011	Research visit (2 weeks) with Dr. Carlo Sala, CNR Institute of Neuroscience & Instituto Neurologico Carlo Besta, Milan, Italy
2006	Research visit (1 month) with Dr. C.C. Hoogenraad, Erasmus Medical Center, Rotterdam, Holland
2002-2005	Postdoctoral Associate with Prof. Morgan Sheng, Picower Center for Learning and Memory, Massachusetts Institute of Technology and Howard Hughes Medical Institute, Cambridge, MA, USA
2000	Research training (1 month) with Dr. J. Guzowski, ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, USA
1997-2001	Research training (7 months) with Prof. J. Mallet, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs (LGN), UMR 9923, Centre National de la Recherche Scientifique, Paris, France
1996-2002	PhD student (until 2001) and Postdoctoral Associate (until May 2002) with Prof. L. Kaczmarek, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1995-1996	Master's degree, Prof. P. Węgleński, Department of Genetics, Warsaw University, Poland

Fellowships and Awards

2011	Prime Minister Award for habilitation thesis
2009	2nd Division (Biological Sciences) of Polish Academy of Sciences Award for series of publications on MMP9 (together with teams of Prof. Kaczmarek and Dr. Wilczynski)
2005	Konorski Award for best publication of 2004 in the field of neuroscience (Kowalczyk et al., J Cell Biol, 2004, 167:209-213), Polish Neuroscience Society and Polish Academy of Sciences
2002	Prime Minister Award for PhD thesis
2001	Foundation for Polish Science National Scholarship for Young Investigators (1 year scholarship)
2000	EMBO Short-Term Fellowship
1999	Polish Network for Cell and Molecular Biology UNESCO/PAN Scholarship
1997	French Government Scholarship

Membership in Scientific Societies, Organizations, and Panels

2011	Neurobiology Committee of the Polish Academy of Sciences, Member

Awards, Honors and Titles (Lab members - 2012)

2012	The Nencki Institute Scientific Council distinction for PhD thesis, M. Perycz
2012	PhD in Molecular Biology, Nencki Institute, M. Perycz
2012	The Nencki Institute Scientific Council distinction for PhD thesis, A. Malik
2012	PhD in Molecular Biology, Nencki Institute, A. Malik
2012	Mazovia 1-year PhD Scholarship A. Urbańska
2012	Homing Plus from Foundation for Polish Science, E. Liszewska
2012	Fuga from National Science Center, B. Tarkowski
2011	EMBO Long-Term Scholarship for postdoctoral training at Broad Institute, Ł. Świech
2011	Selection for "Top Innovator 500" Ministerial Program, I. Cymerman
2011	The Nencki Institute Scientific Council distinction for PhD thesis, Ł. Świech
2011	PhD in Molecular Biology, Nencki Institute, Ł. Świech
2011	Mazovia 1-year PhD Scholarship, M. Urbanska

Selected publications

Publications in 2010-2012

- **Malik AR, Urbanska M, Gozdz A, Swiech LJ,** Nagalski A, **Perycz M, Blazejczyk M, Jaworski J**. Cyr61, a matricellular protein, is needed for dendritic arborization of hippocampal neurons. J Biol Chem, 2013; Jan 28. [Epub ahead of print]
- **Malik AR, Urbanska M, Macias M, Skalecka A, Jaworski J**. Beyond control of protein translation: What we have learned about the non-canonical regulation and function of mammalian target of rapamycin (mTOR). Biochim Biophys Acta - Proteins and Proteomics, 2012 [Epub ahead of print]
- Knapska E#, **Macias M,** Mikosz M, Nowak A, Owczarek D, Wawrzyniak M, **Pieprzyk M, Cymerman IA,** Werka T, Sheng M, Maren S, **Jaworski J**#, Kaczmarek L#. Functional anatomy of neural circuits regulating fear and extinction. Proc Natl Acad Sci, 2012; 109(42):17093-8 # - corresponding authors
- **Urbanska M, Gozdz A, Swiech LJ, Jaworski J.** Mammalian target of rapamycin complex 1 (MTORC1) and 2 MTORC2) control the dendritic arbor morphology of hippocampal neurons. J Biol Chem, 2012; 287(36):30240-56
- **Urbanska M, Swiech L, Jaworski J.** Developmental plasticity of the dendritic compartment: focus on the cytoskeleton. Adv Exp Med Biol, 2012; 970:265-84
- Kapitein LC, Yau KW, Gouveia SM, van der Zwan WA, Wulf PS, Keijzer N, Demmers J, **Jaworski J**, Akhmanova A, Hoogenraad CC. NMDA Receptor Activation Suppresses Microtubule Growth and Spine Entry. J Neurosci, 2011; 31(22):8194-209
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- **Perycz M**, **Urbanska AS**, **Krawczyk PS**, **Parobczak K**, **Jaworski J.** Zipcode Binding Protein 1 Regulates the Development of Dendritic Arbors in Hippocampal Neurons. J Neurosci, 2011; 31(14):5271–85
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Other selected publications

- **Jaworski J**, Kapitein LC, Montenegro Gouveia S, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity, Neuron, 2009; 61:85-100
- **Swiech L, Perycz M, Malik A, Jaworski J**. Role of mTOR In physiology and pathology of the nervous system. Biochim Biophys Acta, 2008; 1784:116-132
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- ***Jaworski J**, Biederman I, Lapinska J, Szklarczyk AW, Figiel I, Konopka D, Nowicka D, Filipkowski RK, Hetman M, Kowalczyk A, Kaczmarek L. Neuronal excitation driven and AP-1-dependent activation of timp1 gene expression in rodent hippocampus. J Biol Chem, 1999; 274: 28106-12

*no IIMCB affiliation

Description of Current Research

The research of our team concentrates on the role of the protein kinase mammalian target of rapamycin (mTOR) in the control of proper neuronal morphology in health and disease. Establishing proper neuronal morphology is required for proper brain function. Therefore, the mechanisms of axon targeting, dendritic arbor patterning, proper cell contact formation, and the maintenance of neuronal connectivity plasticity are at the center of interest of molecular neurobiology. Dendrites are the main site of information input into neurons, and dendritic arbor shape is one of the crucial factors that determine how signals that originate from individual synapses are integrated. Several neurodevelopmental pathologies are characterized by abnormalities in dendritic tree structure. Dendritic arbor development is a multistep process that depends on, among other factors, mTOR, a serine/threonine protein kinase known to merge extracellular instructions with information about cellular metabolic resources and control the rate of anabolic and catabolic processes accordingly. In neurons, mTOR has been implicated in neuronal differentiation, axon elongation and directional movements, spinogenesis, long-term synaptic plasticity, and learning and memory. In neurons, mTOR is hypothesized to act primarily by controlling protein

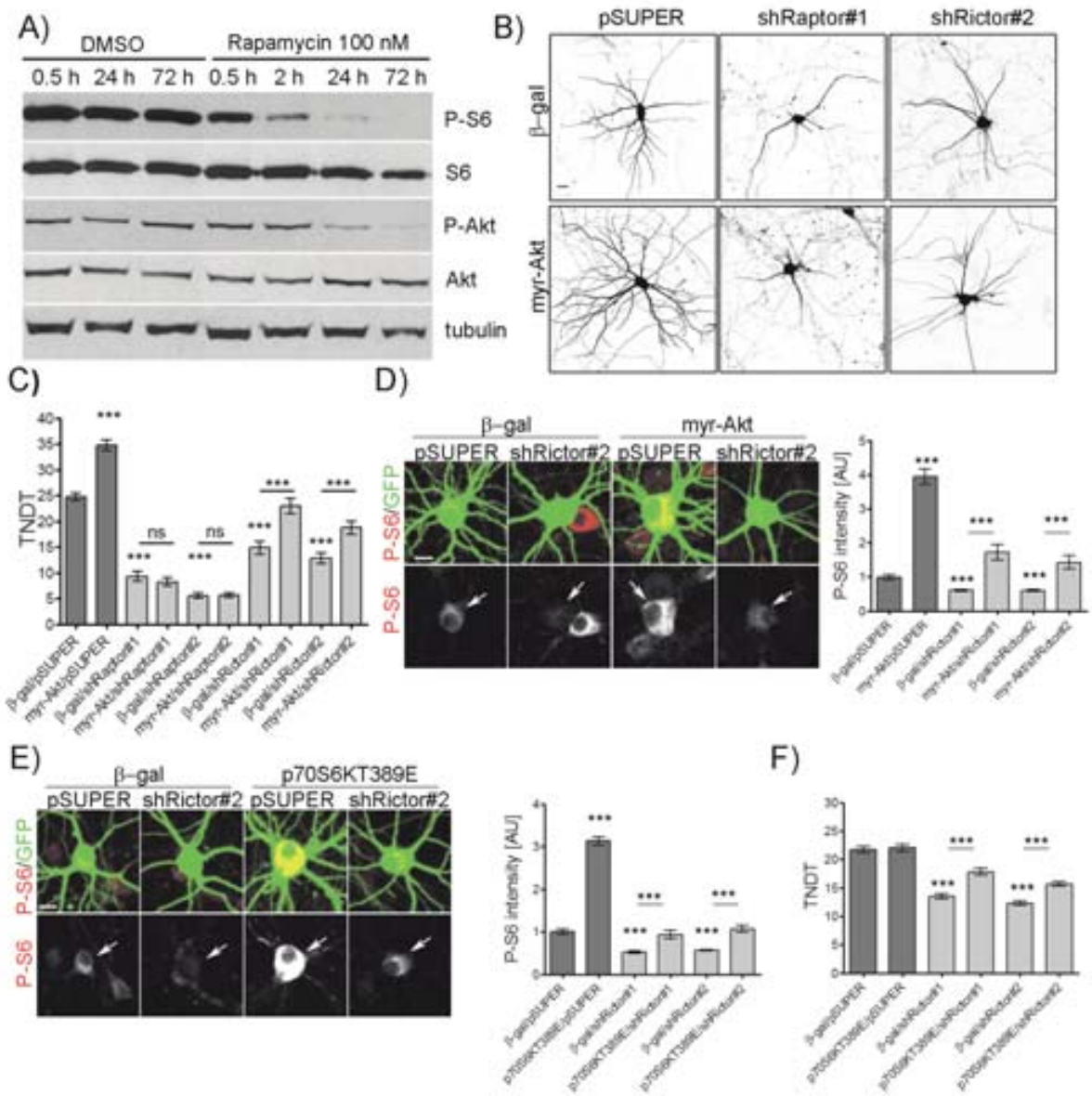

Fig. 1. mTORC1 controls mTORC2 activity in developing neurons. (***A***) Prolonged rapamycin treatment inhibits mTORC2 in neurons. Western blot analysis of protein lysates obtained from hippocampal neurons cultured *in vitro* and treated with 100 nM rapamycin on DIV (day in vitro) 8 for 0.5, 2, 24, and 72 h. P-S6 (Ser235/S236) and P-Akt (S473) were checked as a canonical effectors of mTORC1 and mTORC2, respectively. (***B***) Rictor knockdown inhibits dendritic growth, which can be rescued by Akt activation. Representative images hippocampal neurons cultured *in vitro,* co-transfected on DIV8 for 5 days with either EF*α*-*β*-gal (control) or myr-Akt and control pSUPER vector or PSUPER-shRaptor#1, - shRaptor2, pSUPER-shRictor#1 or -shRictor#2. Neuronal morphology was visualized by co-transfected GFP. Representative images of neurons transfected as indicated. (***C***) Total number of dendritic tips (TNDT) of hippocampal neurons transfected as indicated. (***D***) Rictor knockdown decreases mTORC1 signaling in Akt-dependent manner. Hippocampal neurons cultured *in vitro* were transfected on DIV8 for 3 days with either EF*α*-*β*-gal (control) or myr-Akt and control pSUPER vector or pSUPER that encoded shRNA against Rictor (shRictor#1, shRictor#2). The cells were co-transfected with a GFP-coding vector for visualization. Cells were stained for P-S6 (Ser235/S236) and averaged intensity of cell body immunostaining was measured. (***E***) Hippocampal neurons cultured *in vitro* were transfected on DIV8 for 3 days with either EF*α*-*β*-gal (control) or p70S6KT389E and control pSUPER vector or pSUPER that encoded shRNA against Rictor (shRictor#1, shRictor#2). The cells were co-transfected with a GFP-coding vector for visualization. Cells were stained for P-S6 (Ser235/S236) and averaged intensity of cell body immunostaining was measured. (***F***) TNDT of neurons transfected as in ***E***. Photos by Malgorzata Urbańska; for more details please refer to Urbanska et al., 2012, *J.Biol.Chem.*, 287:30240-56.

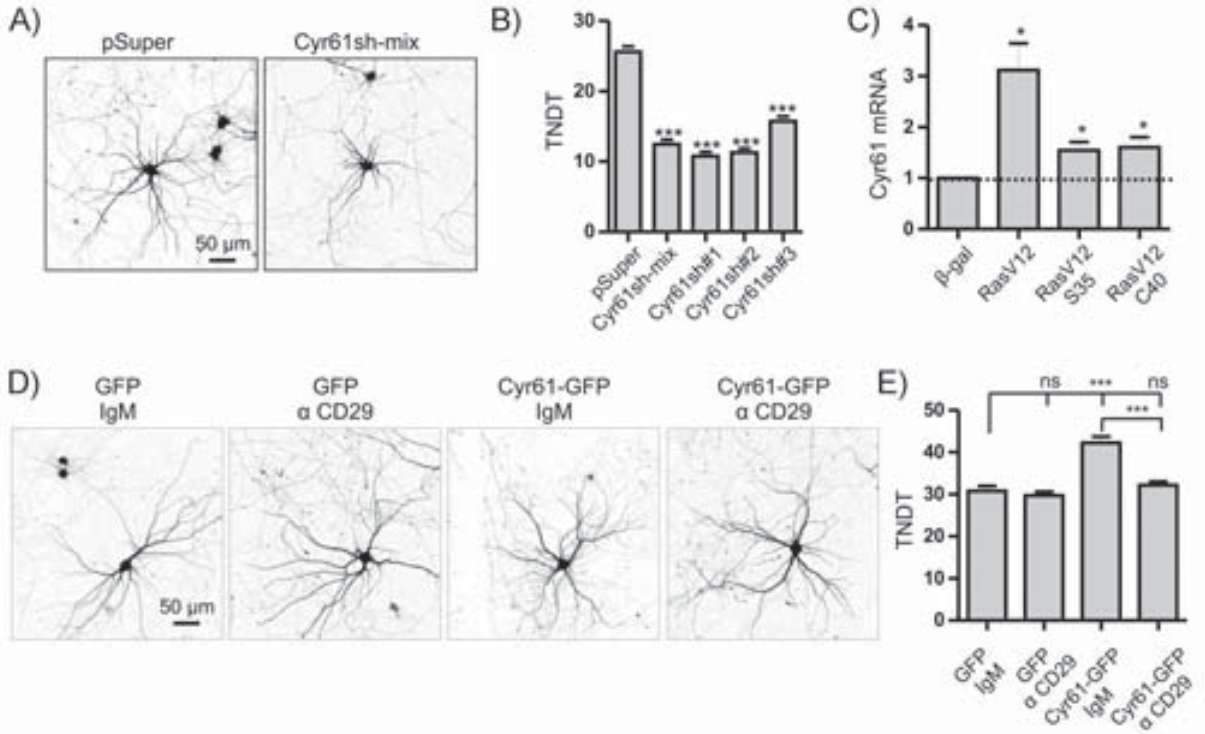

Fig. 2. Cyr61, a matricellular protein controls dendritic arbor growth. (***A, B***) Knockdown of Cyr61 in developing neurons simplifies dendritic tree morphology. (***A***) Representative images of cultured *in vitro* rat hippocampal neurons transfected on DIV8 for 4 days with pSuper vector (control) or plasmids that encode pool of Cyr61 shRNAs (Cyr61sh-mix). The GFP-encoding plasmid was cotransfected for the visualization of transfected cells. (***B***) Total number of dendritic tips (TNDT) of hippocampal neurons transfected as indicated. (***C***) Ras-ERK and Ras-PI3K signaling pathways increase Cyr61 mRNA levels. Results of RT-qPCR analysis of Cyr61 mRNA levels in cortical neurons nucleofected with either β-gal (control) or Ras mutant-encoding vectors. Cyr61 mRNA levels were quantified relative to GAPDH and compared with controls in each experiment. (***D, E***) Cyr61 overexpression induces dendritic tree growth in a β1-integrin dependent manner. (***D***) Representative images of hippocampal neurons transfected on DIV8 for 6 days with GFP- or Cyr61-GFP-encoding plasmids. mRFP-encoding plasmid was cotransfected for the visualization of transfected cells. Anti-CD29 (anti-β1-integrin) or control IgM antibody was added to the medium 2 and 4 days after transfection. (***E***) TNDT of neurons transfected as in ***D***. Photos by Anna Malik; for more details please refer to Malik et al., 2013, *J.Biol.Chem.*, Jan 28. [Epub ahead of print]

translation, including local protein synthesis in dendrites. Studies in different model systems (e.g., yeast, fruit flies, and cultured non-neuronal mammalian cells) strongly imply the involvement of mTOR in additional cellular processes, such as transcription, membrane trafficking, mitochondrial function, lipid metabolism, autophagy, and cytoskeleton dynamics. Thus, considering the key role that mTOR plays in cell physiology, unsurprising is that mTOR signaling is disturbed under various neuropathological conditions. Altered mTOR activity has been reported in brain tumors, tuberous sclerosis (TSC), cortical dysplasia, and neurodegenerative disorders. However, in cases of either physiological processes or neuropathology, our knowledge of the molecular events downstream of mTOR, other than protein translation, is rather limited. We believe that expanding such knowledge is crucial for understanding the molecular biology of neurons and assessing the benefits and risks of the clinical use of mTOR inhibitors. Thus, our goal is to determine the mTOR-dependent proteins and cellular processes involved in neuronal development. For the past few years, our research has developed in two main areas:

1. Identifying mTOR partners and regulated proteins involved in the processes of dendritic branching and synapse formation and stabilization.
2. Establishing a link between local protein translation and physiological dendritic arbor development.

In 2012, we continued our work within both of these areas. Below we describe our research that led to two important discoveries: (*i*) finding a new mode of action of mTOR complexes in neurons and (*ii*) description of neuronal functions of matricellular protein Cyr61. We also succeeded in establishing an animal model to study dendritogenesis in the intact brain. Using this model, we demonstrated a role for mTOR in dendrite growth *in vivo*. In collaboration with Dr. Knapska and Prof. Kaczmarek, we finalized a project that was initiated by Dr. Jaworski during his postdoctoral training. Specifically, we showed that our transgenic rat that expresses PSD95: Venus fusion protein under the control of a c-*fos* promoter can be used as a novel tool for the neuroanatomical tracing of active neuron connections.

mTORC1 controls mTORC2 activity in developing neurons

Our previous studies demonstrated that rapamycin, an inhibitor of mTOR, is able to prevent the dendritic arborization of neurons grown *in vitro*. However, over past few years, the effects of rapamycin on any biological process has been far from obvious. mTOR forms two functionally distinct complexes in mammalian cells: mTORC1 and mTORC2. These complexes share some protein components, but their distinct activities are defined by their unique components: Raptor, Rictor, and mSin1. mTORC1 contains Raptor and is the actual target of rapamycin. mTORC2 contains Rictor and mSin1 and is considered resistant to acute treatment with this drug. Nevertheless, in several non-neuronal cell types, long-term rapamycin application led to mTORC2 inhibition. This issue has not yet been methodically addressed in neurons but raised concerns about which mTOR complex is involved in the control of dendritic arbor growth. Our previous studies indicated that mTORC1 may be involved but did not rule out the involvement of mTORC2. Thus, we decided to more closely investigate this issue because taking a step backward was crucial for understanding neuronal mTOR

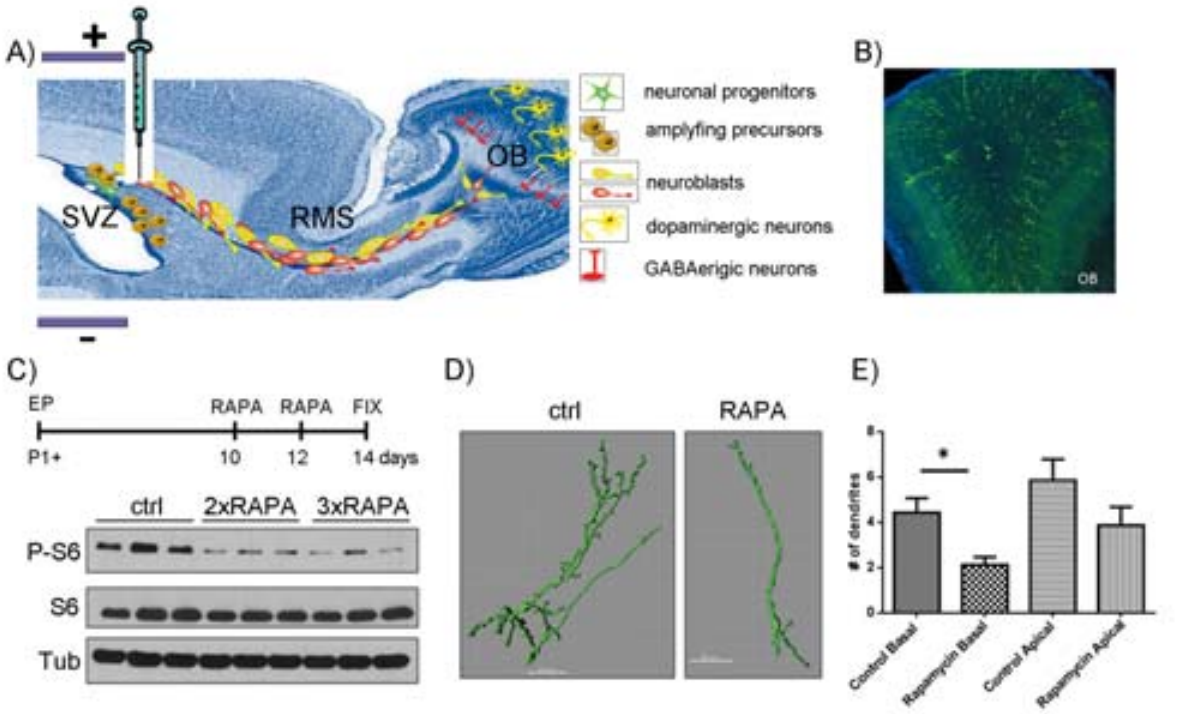

Fig. 3. An animal model to study dendritogenesis *in vivo*. (***A***) Cartoon presenting principles of neuroblast migration trough SVZ-RMS-OB and *in vivo* electroporation technique. (***B***) Representative confocal image of GFP electroporated neurons (green) in OB two weeks post electroporation (***C***) Scheme of an experiment testing role of mTOR in dendritic branching *in vivo* (upper panel) and Western blot analysis of effectiveness of *in vivo* rapamycin (RAPA) application on a canonical mTORC1 target – ribosomal protein S6 in OB (lower panel). (***D***) Representative 3D reconstructions of GFP-electroporated OB neurons from control and rapamycin treated animals. (***E***) Quantification of a number of dendrites of electroporated OB neurons from control and rapamycin-treated animals. Cartoons and photos by Agnieszka Skałecka.

functions. Our studies first showed that mTORC2 in neurons is indeed inhibited by prolonged rapamycin treatment (Fig. 1). Thus, we were forced to find an alternative approach to distinguish the actions of mTORC1 and mTORC2 in neurons. To achieve this, we used short-hairpin RNA (shRNA) technology to target Raptor and Rictor and knock down mTORC1 and mTORC2 activity, respectively. Using this approach, we provided evidence that both mTOR complexes are crucial for the proper dendritic arbor morphology of hippocampal neurons (Urbanska et al., 2012). These two complexes are required for dendritic development, both under basal conditions and upon the induction of mTOR-dependent dendritic growth (Urbanska et al., 2012). We also identified Akt as a downstream effector of mTORC2 needed for proper dendritic arbor morphology (Urbanska et al., 2012). However, we also made a surprising discovery, in which mTORC2 was found to be an upstream regulator of mTORC1 in neuronal cells (Fig. 1). This observation conflicted with observations of mouse embryonic fibroblasts and several other non-differentiated and actively dividing cells that clearly showed that either knockout or knockdown of Rictor did not decrease the activity of p70S6K, a kinase that is a direct effector of mTORC1. However, in neurons, we observed substantially decreased p70S6K activity in cells with silenced Rictor. Moreover, the overexpression of constitutively active p70S6K was sufficient to substantially rescue dendritic arbor retardation caused by mTORC2 inhibition (Fig. 1). Our finding that mTORC2 can regulate mTORC1 was recently confirmed by others in hepatocytes, suggesting that this mechanism operates in terminally differentiated cells.

Cyr61, a matricellular protein, controls mTOR-dependent dendritic arbor growth

Since the inception of the Laboratory of Molecular and Cellular Neurobiology, we have been trying to identify proteins that are involved in mTOR-dependent dendritic arborization. We performed a medium-scale shRNA library screen, the results of which have already been described in previous annual reports. In 2012, we focused on one of our positive hits, matricellular protein cysteine rich 61 (Cyr61). Matricellular proteins are secreted proteins that are closely associated with the extracellular matrix (ECM). Rather than being structural components of the ECM, however, such proteins contribute to cell signaling by acting as modulators of cell surface receptors. For example, Cyr61 is a ligand of different integrins. Through these interactions, it regulates cell adhesion, migration, proliferation, survival, apoptosis, differentiation, gene expression, and senescence, depending on the cell type and particular integrin involved. Although Cyr61 is mostly known for its roles in various non-neuronal cells, it is also expressed in the developing nervous system and neuron-like cells. Nonetheless, knowledge about the function of this protein in neurons is very limited. Because Cyr61 can be expressed in an mTOR-dependent manner, Dr. Anna Malik studied its role in the context of the mTOR-dependent dendritic arborization of neurons grown *in vitro* (Malik et al., 2013). Together with other Laboratory of Molecular and Cellular Neurobiology members and with the help of Andrzej Nagalski from the Kuznicki laboratory, Dr. Malik showed that (*i*) *cyr61* is expressed in developing neurons in a trophic factor-, Ras-, extracellular signal-regulated kinase-, and phosphoinositide 3 kinase-dependent manner, and (*ii*) knockdown of *cyr61* prevented dendritic growth under basal conditions and dendritic growth induced by insulin, Ras, and PI3K (Fig. 2). Interestingly, we also showed that Cyr61 overexpression induced dendritic growth in a β1-integrin-dependent manner (Fig. 2). This may suggest the existence of a positive feedback loop, in which Cyr61 sustains PI3K-mTOR activity itself because it is a known inducer of this pathway.

Development of an animal model to study dendritogenesis and spinogenesis *in vivo*

During the past few years, we identified several potential mechanisms through which mTOR can contribute to neuronal development, including protein synthesis (Jaworski et al., 2005) and the control of cytoskeleton dynamics (Swiech et al., 2011). Nevertheless, these findings require confirmation *in vivo*. We decided to introduce a new research model in our laboratory: the integration of newly born neurons to the olfactory bulb (OB) in rodents. The OB is one of two regions in the adult brain where new functional neurons are continuously incorporated into preexisting neuronal circuits. The OB is a destination for neuronal progenitors born in the subventricular zone (SVZ) that then migrate through the rostral migratory stream (RMS). Therefore, the SVZ-RMS-OB pathway is a unique system to study the molecular mechanisms of neurogenesis, neuronal development, and neuronal network reconstruction *in vivo*. Owing to novel technology called *in vivo* electroporation (Fig. 3), the SVZ-RMS-OB system is easy for fast genetic modification. In 2011, Agnieszka Skalecka, a PhD student in the laboratory, successfully established *in vivo* electroporation conditions. In 2012, using this protocol, she showed that the rapamycin-driven inhibition of mTOR retards the dendritic arbors of glomerular and periglomerular neurons in the OB (Fig. 3). This was an important step in the investigation of the specific roles of mTORC1, mTORC2, and their selected targets, especially those involved in cytoskeleton dynamics and membrane trafficking, in dendritogenesis and spinogenesis *in vivo*.

c-Fos-PSD95:Venus-Arc transgenic rat: a modern tool to trace synapses through active neuronal circuits

Understanding the dynamics of neuronal circuits requires new and advanced tools for tracing neuronal connectivity under various functional states of neuronal networks. As a postdoc in the Morgan Sheng Lab, Dr. Jaworski began constructing a transgenic animal that allows the visualization of synapses of active neurons. However, only last year was this project finalized, thanks to collaborations with the Knapska Lab and Kaczmarek Lab from the Nencki Institute. We generated a novel tool (i.e., c-Fos-PSD95:Venus-Arc, the "Venus rat") to visualize synapses of activated neurons. Postsynaptic density-95 (PSD-95) is a major component of postsynaptic densities. The *Arc* untranslated region contains dendrite-localizing sequences, and the c-*fos* promoter is induced by neuronal activity. Therefore, Venus rats allow the dendrites and synapses of activated neurons to be visualized with fluorescent tags. As a proof-of-concept for our strategy, we cultured neurons from transgenic rats and analyzed the expression inducibility and cellular distribution of PSD95:Venus. As shown in Fig. 4, PSD-95:Venus could not be detected in neurons cultured under basal conditions. However, increased neuronal activity resulted in the robust induction of transgene-encoded protein and PSD-95:Venus incorporation into postsynaptic sites. Once we confirmed our transgenic model in cultured cells, we examined whether fear conditioning induces transgene expression and drives transgenic protein into the synaptodendritic compartment in behaving animals. As shown in Fig. 4, we observed PSD95:Venus expression in the amygdala as soon as 2 h after fear conditioning, whereas it was not detectable in control Venus rats. Importantly, strong expression of the transgene was observed in cells stained for endogenous c-Fos, further validating our novel experimental animal model. The expression of PSD-95:Venus persisted up to 24 h after training, which is much longer than the expression of endogenous c-Fos, thus allowing us to track the history of individual neuron activation. Using this animal model in combination with anterograde axonal transport tracers, we were able to show the functional anatomy of neural circuits that regulate fear and its extinction, providing a framework for therapeutic manipulations of these circuits (Knapska et al., 2012).

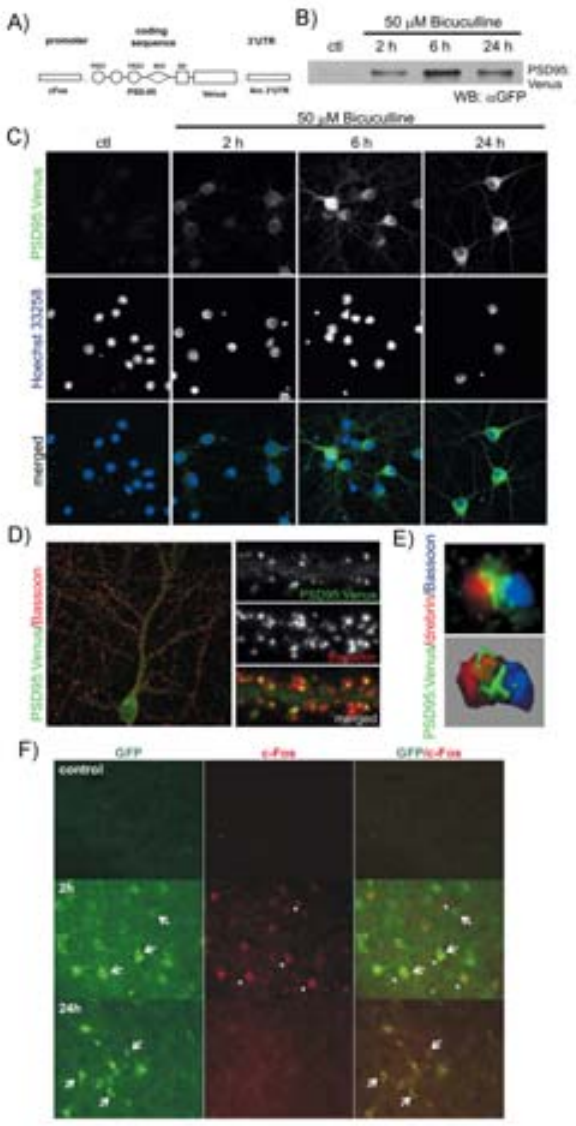

Fig. 4. c-Fos-PSD95:Venus-Arc transgenic rat: a modern tool to trace synapses through active neuronal circuits. (***A***) Schematic representation of a cFos-PSD-95:Venus-Arc transgenization cassette. (***B***) Western blot analysis of PSD-95:Venus expression in transgenic cortical neurons cultured *in vitro* under basal conditions and after increased neuronal network activity (50 μM bicuculline treatment). (***C***) Immunofluorescence analysis of PSD-95:Venus expression in transgenic hippocampal neurons cultured *in vitro* under basal conditions and after increased neuronal network activity (50 μM bicuculline treatment). (***D***) Representative image of double immunofluorescent labeling of transgenic neuron (6 h post bicuculline) for Venus (with anti-GFP antibody) and the presynaptic marker, Bassoon, showing localization of PSD-95:Venus to synapses. (***E***) Three dimensional reconstruction of a single synapse of a transgenic neuron immunofluorescently stained for Venus, Bassoon and the postsynaptic protein, drebrin. Upper panel: raw image, bottom panel: 3D reconstruction. (***F***) Expression of the fusion PSD-95:Venus protein (Venus-positive, green) was visible in the amygdala 2h after fear conditioning training, whereas it was not detectable in control (non-shocked) animals. The cells which expressed PSD-95:Venus protein were also positive for endogenous c-Fos (red). The PSD-95:Venus signal was still visible 24 h after fear conditioning, when endogenous c-Fos staining was already absent. Photos by Jacek Jaworski, Matylda Macias and Ewelina Knapska; for more details please refer to Knapska et al., 2012, *Proc Natl Acad Sci U S A.*, 109:17093-8.

Walker carcinosarcoma expressing the actin binding peptide Lifeact. Cells display blebs (cyan cells) or lamellipodia (yellow cells).
Author: Martin Bergert.

Laboratory of
Cell Cortex Mechanics MPG/PAN

(located at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden)

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Andrea Pereira, PhD
Matthew B. Smith, PhD (from Feb. 2013)

Junior Researchers:
Martin Bergert, MSc
Andrew G. Clark, BSc
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Head of Laboratory of Cell Cortex Mechanics MPG/PAN

Ewa Paluch, PhD

Degrees:

2005	PhD in Biophysics, University Paris 7, Paris, France
	2001 DEA (Master's degree) "Interfaces Physique-Biologie," University Paris 7 (rank: 1st), Paris, France
2000	Agrégation of Physics
1999	Maîtrise (equivalent to BSc) in Physics, Ecole Normale Supérieure de Lyon, France
1998	Licence in Physics, Ecole Normale Supérieure de Lyon, France

Research Training:

2001-2005	PhD studies at the Institut Curie, Paris, France
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1999	Maîtrise (BSc) research project in Particle Physics, CERN, Geneva, Switzerland
1998	Licence (part of BSc) research project in Relativistic Astrophysics, Paris-Meudon Observatory, France

Professional Employment:

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2005	Scientist position at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Honors and Fellowships :

2005	Joint MPI-CBG/PAN group leader at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
2004-2005	PhD scholarship, Ligue Nationale Contre le Cancer, France
2001-2004	PhD scholarship, CNRS, France
2000	Agrégation in Physics (French national competition, rank: 6th)
1998-2001	Full salary from Ecole Normale Supérieure de Lyon, France (recruitment by national competition)
1995	Prize of Scientific and Technical Vocation of Girls, awarded by the Regional Delegation for Women Rights, region of Paris, France

Grants

- From 2013 *Medical Research Council laboratory core funding
- 2013-2018 *European Research Council, "The inherent morphological potential of the actin cortex and the mechanics of cell shape control during cell division"; EUR 1,500,000
- 2009-2012 Polish Ministry of Science and Higher Education, International Project Grant (MPG Program), "The role of cell cortex mechanics in cell motility" (454/N-MPG/2009/0); PLN 4,692,929
- 2009-2010 *Deutsche Forschungsgemeinschaft (DFG) grant to Carl-Philipp Heisenberg (MPI-CBG, Dresden) and Ewa Paluch, "Analysis of the formation and function of different cell protrusion types during cell migration in vivo" (PA 1590/-1); EUR 70,600 + 1 PhD position/team
- 2008-2011 *Human Frontier Science Program (HFSP) Young Investigators' Grant to Guillaume Charras (UCL, London, UK), Guillaume Romet-Lemonne (CNRS, Gif-sur-Yvette, France), Philippe Roux (IRIC, Montreal, Canada), and Ewa Paluch, "Interplay between mechanical and biological mechanisms during cell cortex assembly" (RGY 67/2008); $337,500/team
- 2006-2009 Polish-German Special Grant, "The role of cell cortex contractility in the establishment and positioning of the cleavage furrow" (JRGP/37/2005), Max Planck Society (MPG) – Polish Academy of Sciences (PAN) MPI-CBG Junior Research Program – Laboratory of Cortex Movement and Cell Division MGP/PAN; PLN 3,024,200

*no IIMCB affiliation

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- Maître JL, Berthoumieux H, Krens SF, Salbreux G, Jülicher F, **Paluch E** (#1), Heisenberg CP (#1). Adhesion functions in cell sorting by mechanically coupling the cortices of adhering cells. Science. 2012 Oct 12;338(6104):253-6 (1) co-corresponding
- Green RA, **Paluch E**, Oegema K. Cytokinesis in animal cells. Annu Rev Cell Dev Biol, 2012; 28:29-58
- **Bergert M, Chandradoss SD**, Desai RA, **Paluch E**. Cell mechanics control rapid transitions between blebs and lamellipodia during migration. Proc Natl Acad Sci U S A, 2012;109(36):14434-9
- Salbreux G, Charras G, **Paluch E**. Actin cortex mechanics and cellular morphogenesis. Trends Cell Biol, 2012; 22(10):536-45
- Goudarzi M, Banisch TU, Mobin MB, Maghelli N, Tarbashevich K, Strate I, van den Berg J, Blaser H, Bandemer S, **Paluch E**, Bakkers J, Tolić-Nørrelykke IM, Raz E. Identification and Regulation of a Molecular Module for Bleb-Based Cell Motility. Dev Cell, 2012; 23:210-8
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- **Clark AG**, **Paluch E**. Mechanics and regulation of cell shape during the cell cycle. Book chapter in "Cell Cycle in Development", Ed. JZ Kubiak, Results Probl Cell Differ (Springer-Verlag), 2011; 53:31-73
- **Diz-Munoz A**, Krieg M (1), **Bergert M**, Ibarlucea-Benitez I, Muller DJ, **Paluch E** (1), Heisenberg CP (1). Control of directed cell migration in vivo by membrane-to-cortex attachment. PLoS Biol. 2010 Nov 30;8(11):e1000544 (1) co-corresponding
- **Tinevez JY, Schulze U**, Salbreux G, **Roensch J**, Joanny J-F, **Paluch E**. Role of cortical tension in bleb growth. Proc Natl Acad Sci USA, 2009; 106:18581-86
- **Paluch E**, Heisenberg CP. Biology and Physics of Cell Shape Changes in Development (review). Curr Biol, 2009; 19:R790-799
- Charras G, **Paluch E**. Blebs lead the way: how to migrate without lamellipodia (review). Nat Rev Mol Cell Biol, 2008; 9:730-736
- **Paluch E**, Van der Gucht J, Sykes C. Cracking up: symmetry breaking in cellular systems. J Cell Biol, 2006; 175:687-692
- *__Paluch E__ (1), van der Gucht J (1), Joanny J-F, Sykes C. Deformations in actin comets from rocketing beads. Biophys J, 2006; 91:3113-22 (1) shared authorship
- *__Paluch E__, Sykes C, Prost J, Bornens M. Dynamic modes of the cortical actomyosin gel during cell locomotion and division. Trends Cell Biol, 2006; 16:5-10
- *__Paluch E__, Piel M, Prost J, Bornens M, Sykes C. Cortical actomyosin breakage triggers shape oscillation in cells and cell fragments, Biophys J, 2005; 89:724-33
- *van der Gucht J, **Paluch E**, Plastino J, Sykes C. Stress release drives symmetry breaking for actin-based movement, Proc Natl Acad Sci USA, 2005; 102:7847-52.

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Publications (other than scientific articles)

- Articles about the history of words related to physics and biology for Dictionnaire Culturel de la Langue Francaise (2005) directed by Alain Rey, publisher: le Robert (informations: http://www.lerobert-dictionnaireculturel.com/)
- Paluch E, Ramspacher A. (1998) Electromagnetisme, 2eme annee, collection Puissance Prepas, publisher: Breal (methods and corrected exercises for 2nd year Physics students)

Research

The main focus of our group's research is to investigate the principles that underlie cellular morphogenesis. Cell shape is ultimately defined by cellular mechanical properties and the cell's physical interactions with its environment. Biophysical approaches are essential to understand cell shape control (Clark and Paluch, 2011; Paluch and Heisenberg, *Curr Biol*, 2009). We combine biology, quantitative image analysis, and physical modeling to investigate the molecular regulation of cellular mechanical properties and contribution of these properties to cellular deformations. Animal cell shape is determined to a great extent by the actin cortex, a network of actin filaments, myosin, and associated proteins that lie immediately beneath the plasma membrane. The cortex enables the cell to resist externally applied forces and exert mechanical work. As such, it plays a central role in events that involve cell deformation, such as cell division and cell locomotion, and in the pathophysiology of diseases, such as cancer, in which cortical contractility is often dysregulated (Salbreux et al., *Trends Cell Biol,* 2012). Despite its importance, very little is known about cortex composition, assembly, regulation, and mechanics. To address these issues, we developed a number of methods to probe cortex growth dynamics and nanometer-scale organization and directly measure cortical mechanical properties.

Our main focus is on investigating how cortical mechanical properties are determined by the molecular components of the cortex and how these properties are regulated both locally and globally to allow the cell to undergo deformations during cell division and migration. We are particularly interested in blebs, spherical membrane protrusions driven by contractions of the actomyosin cortex. Although blebs are commonly observed during apoptosis, cell spreading, cytokinesis, and migration, their growth and physiological functions are still poorly understood. We investigate the physical and biological mechanisms of bleb formation and study their function during cytokinesis and migration. Our main lines of research are the following:

1. Regulation of cortex assembly and cortex mechanics

Our central aim is to understand how cortical mechanics are regulated and controlled during cellular morphogenesis. Despite the physiological importance of the cortex, cortical composition and regulation are very poorly understood. Together with the laboratories of G. Charras (UCL, London) and P. Roux (IRIC, Montreal), our partners on an HFSP Young Investigator Grant, we developed methods for quantitative studies of cortex assembly using blebs. Blebs are initially devoid of filamentous actin and reassemble a contractile cortex prior to retraction. Thus, they represent an ideal system for investigating *de novo* cortex growth. Our group developed an assay, in which cortex assembly at the surface of blebs induced by laser ablation (Tinevez et al., *PNAS,* 2009) could be precisely monitored semi-automatically (Biro et al., submitted). Moreover, to narrow the list of potential regulators, we developed a protocol to separate blebs from the cell body and isolate them for biochemical studies. The Roux laboratory analyzed the composition of these isolated cortices using mass spectrometry. Together with the Charras laboratory, we performed a functional characterization that identified the main nucleators of the actin cortex (Bovellan et al., in revision).

Molecular regulators determine the mechanical properties of the cortex by modulating the spatial organization and dynamics of the network. However, even the most basic aspects of the architecture of the cortex, such as cortex thickness. The spatial arrangement of actin filaments are very poorly understood. One reason for this is that the thickness of the cortical network is less than 0.5 µm, making observations using conventional optical microscopy difficult. Over the past few years, we have developed a method for measuring cortex thickness. Taking advantage of the fact that the localization of an object is not limited by optical resolution, we used the distance between cortical actin and the plasma membrane as a measure of cortical thickness. We showed that the cortex of mitotic HeLa cells is 190 nm thick. This thickness was tightly regulated during the cell cycle, and the dynamics of actin cortex turnover was an important determinant of thickness (Clark et al., in revision; Fig. 1). We are currently expanding the method to localize essential cortical components within the actin network, seeking to provide a map of the spatial organization of the cortex.

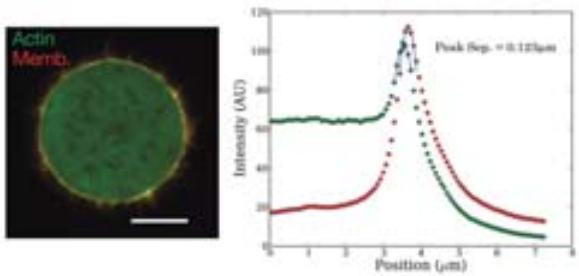


Fig. 1. "Peak-separation" method for cortex thickness measurement. HeLa cell in prometaphase expressing actin-GFP and CAAX-mCherry. Scale bar: 10 µm. The distance between the intensity peaks of cortical actin (green) and of the plasma membrane (red), gives a measure of cortex thickness at a sub-optical-resolution level. Author: Andrew G. Clark.

2. Mechanics of cytokinesis

Cytokinesis relies on the controlled reorganization of the actin cortex. Most previous studies of cytokinetic mechanics focused on force generation in the contractile acto-myosin ring at the cell equator. However, a significant amount of actin and myosin remains at the poles of a dividing cell throughout cytokinesis. We investigated the contribution of this polar cortex to cytokinesis and revealed that polar contractility makes the symmetric shape of the dividing cell intrinsically unstable. Indeed, an imbalance in contractile forces between the two poles can displace the cleavage furrow from its equatorial position. We showed that such instabilities could be observed during cytokinesis and could be amplified by treatments that affect the cortex, leading to shape oscillations and division failure. We proposed a theoretical model that couples cortex tension, turnover, and cell elasticity to quantitatively account for these oscillations (collabouration with Dr. G. Salbreux, MPI-PKS, Dresden). Finally, we showed that blebs, which are commonly observed at the poles of dividing cells, stabilized cell shape by acting as valves that release polar tension. By combining quantitative imaging with physical modeling, this study demonstrated that the shape of a dividing cell was inherently unstable and that polar contractility must be tightly controlled to avoid shape asymmetries and division failure (Sedzinski et al., *Nature*, 2011). In the past year, we have been investigating how the key physical parameters that determine the stability of dividing cells are regulated and quantitatively tested the predictions of our theoretical model (Pereira et al., in preparation).

3. Formation and function of blebs and lamellipodia during cell migration

In three-dimensional environments, bleb-based migration is a widespread alternative to lamellipodial migration and commonly used by cancer cells and during embryonic development (Charras and Paluch, *Nature Rev Mol Cell Biol*, 2008). What determines the type of protrusion formed by a migrating cell and how the various protrusions contribute to migration remain elusive. We developed two model systems to address these issues:

- We are studying cell migration in vivo during Danio rerio (zebrafish) embryonic development (collaboration with the laboratory of Prof. C.P. Heisenberg, IST, Austria). We have shown that mesendoderm progenitor cells migrate during gastrulation using a combination of blebs, lamellipodia, and filopodia. Therefore, they constitute an ideal system for investigating the respective contributions of the different protrusion types to cell migration. We have used various methods to increase the proportion of blebs at the expense of the other protrusion types and have shown that increasing bleb formation slows migration by reducing the directional persistence of the migrating cells (Diz-Munoz et al., *PLoS Biol,* 2010). We also investigated the specific contributions of blebs and lamellipodia to migration by analyzing the dynamics and orientation of the different protrusions with regard to migration direction. We found that zebrafish mesendoderm cells that migrated in vivo alternated phases of directed migration ("run phases") and phases when the cells stall and seemingly explore the environment before reorienting the direction of migration ("tumble phases"). Interestingly, the run phases appeared to be primarily driven by lamellipodia, whereas the tumble phases were associated with blebbing (Diz-Munoz et al., in preparation).
- We have also been investigating the mechanisms that lead to bleb or lamellipodium formation using cultured Walker

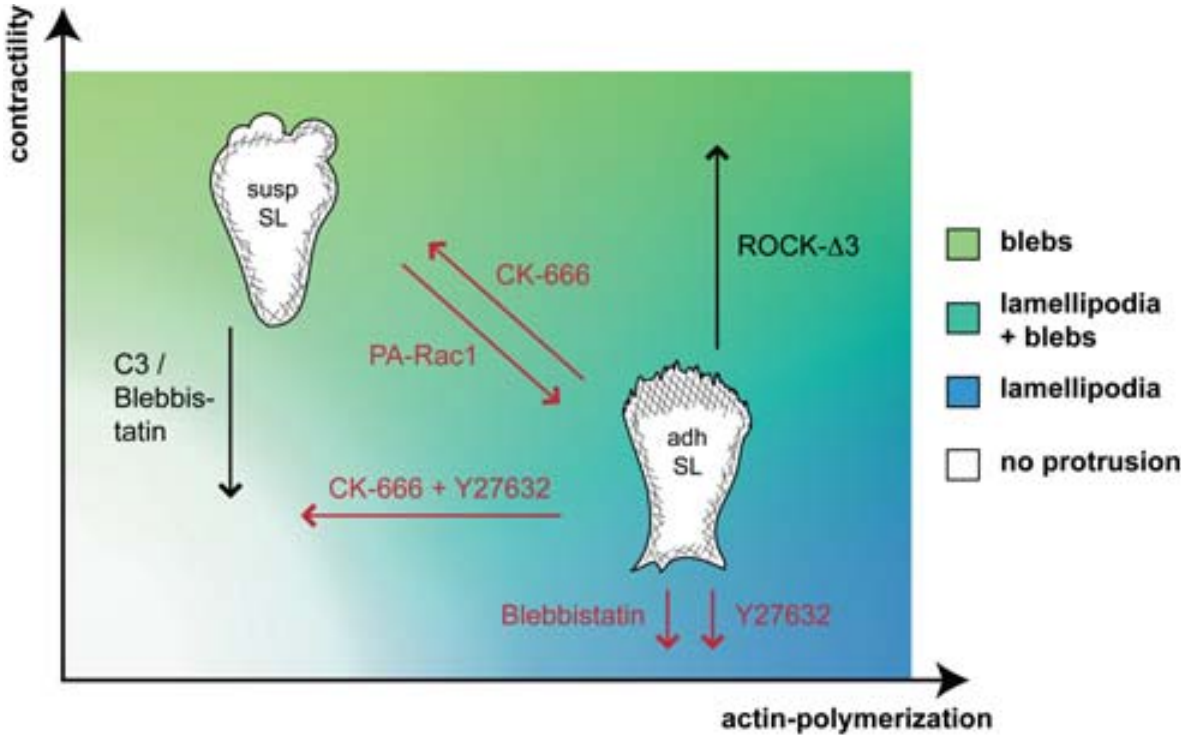


Fig. 2. Changing the balance between polymerization and contractility leads to transitions between blebs and lamellipodia in Walker carcinosarcoma cells. Schematic summary of the effects of various treatments affecting cortex contractility of protrusive actin polymerization. Arrows indicate observed transitions in protrusion types. Red color indicates treatments where immediate transitions could be observed. Figure from (Bergert et al., 2012)

carcinosarcoma cells. These cells can be induced to form either blebs or lamellipodia by varying the culture conditions. We compared the cells that formed lamellipodia to those that formed blebs and characterized the mechanical and molecular requirements for the formation of one or the other protrusion type. We showed that the type of protrusion formed by Walker cells depended on the balance between actomyosin contractility and protrusive actin polymerization. Shifting this balance induced instantaneous transitions between protrusion types. Furthermore, using micropatterning, we found that rapid transitions between blebs and lamellipodia could also be induced by abruptly changing the adhesiveness of the substrate (Fig. 2; Bergert et al., *PNAS,* 2012).

4. Cell-cell adhesion

Finally, we have been investigating the mechanics of cell-cell contact formation in zebrafish mesendoderm progenitor cells. In collaboration with Prof. C.P. Heisenberg (IST, Austria) and Prof. F. Julicher (MPI-PKS, Dresden), we have shown that cell adhesion and cortex tension have very different mechanical functions in controlling progenitor cell-cell contact formation and sorting during zebrafish gastrulation. In contrast to previous models, we showed that the size of the growing cell-cell contact was determined by the contractile tension of the actomyosin cortex. Indeed, as the contact formed, contractile tension decreased at the cell-cell interface, and the size of the contact was the result of the balance of contractile tensions at the contact edge (Fig. 3). In contrast, adhesion was found to make only a minor mechanical contribution to contact expansion. Instead, adhesion was needed to couple the cortices of adhering cells at their contacts, allowing cortex tension to control contact expansion, which in turn drove cell sorting during gastrulation (Maitre et al., *Science,* 2012).

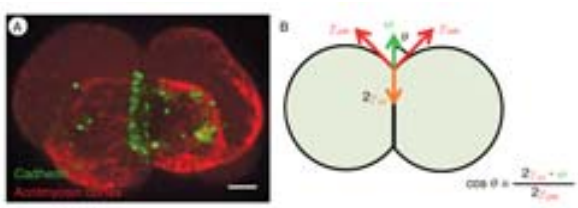
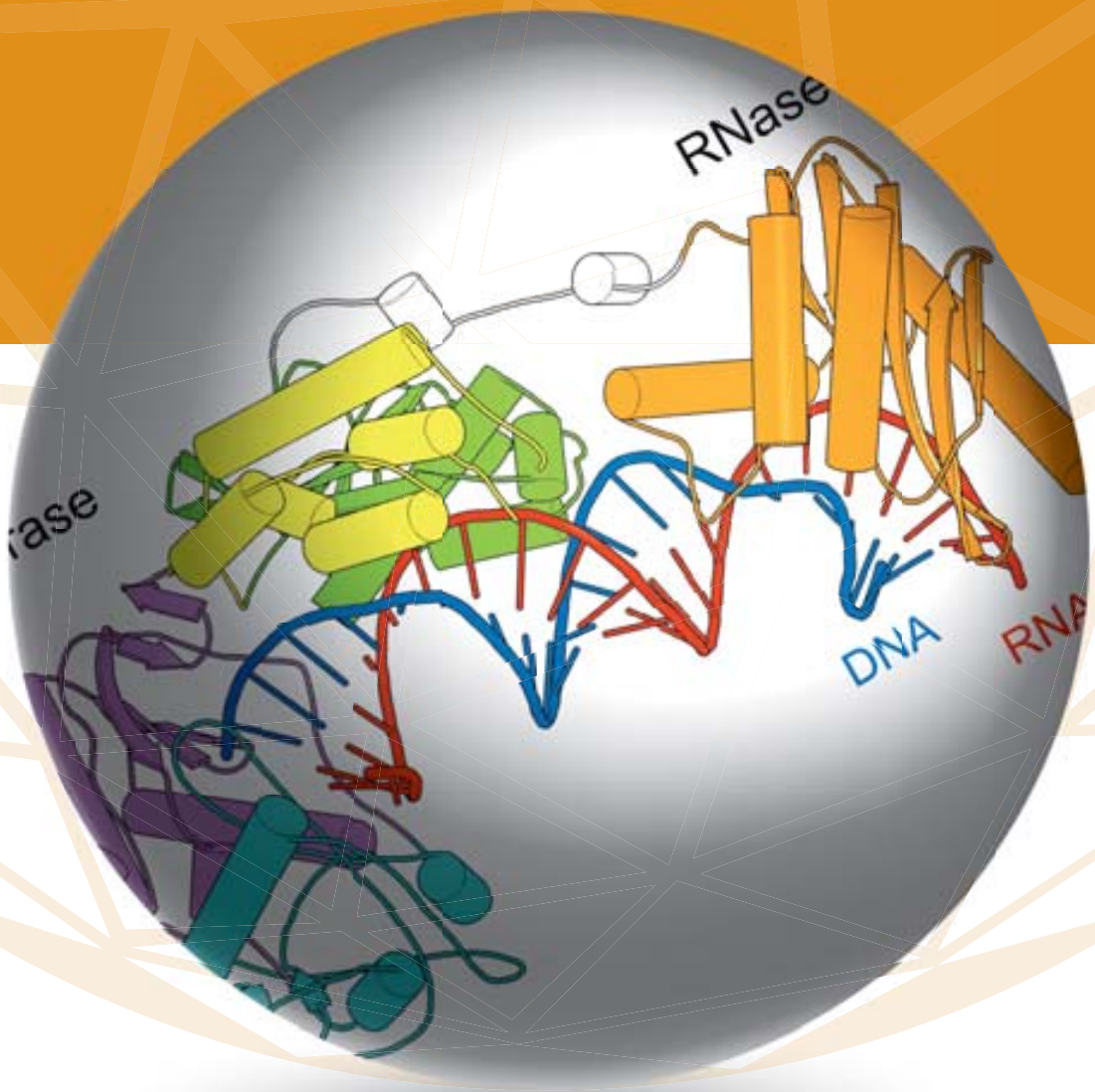


Fig. 3. Forces controlling cell-cell contact formation. Two zebrafish ectoderm progenitor cells forming a cell-cell contact. The cell expresses the adhesion protein cadherin 2 - eGFP (green) and myosin 2 - mCherry (red). Scale bar: 5 µm B. Schematic describing the force balance at the cell-cell contact, which determines the contact angle theta, and thus the shape of the contact. Cortical tension at the cell-cell interface (γ_{cc}) has to balance the cortical tensions at the cell-medium interface (γ_{cm}) and the tension resulting from adhesive complexes (ω). We could experimentally show that ω has a negligible contribution to the force balance. Author: Jean-Léon Maître; figure adapted from (Maitre et al., 2012).



Model of full-length monomeric XMRV RT prepared based on crystal structures and small-angle X-ray scattering data. The DNA polymerase domain is shown in pink, cyan, and yellow. Connection and RNase H domains is green and orange. The RNA/DNA substrate is shown red and blue.

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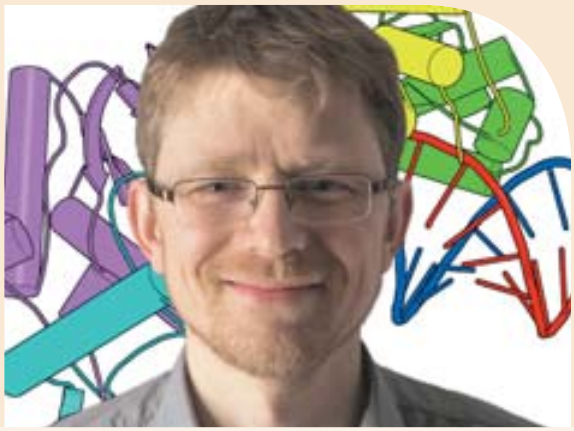

Head of Laboratory of Protein Structure

Marcin Nowotny, PhD

Degrees

2002	PhD *magna cum laude* in Biochemistry, under the supervision of Prof. dr hab. Jacek Kuźnicki, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw
1998	MSc in Organic Chemistry and Biochemistry, Department of Chemistry, Warsaw University

Postdoctoral Training

2003-2008	Postdoctoral Fellow, Wei Yang Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA

Professional Employment

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Honors, Prizes, Awards

2012	HHMI Early Career Scientist Award
2011	ERC Starting Grant
2007	EMBO Installation Grant
2007	Wellcome Trust Senior Research Fellowship
2003	Prime Minister's Award for PhD thesis
2001, 2002	Annual Stipend for Young Scientists, Foundation for Polish Science

Selected publications

- **Nowak E, Potrzebowski W**, Konarev P, Rausch J, Bona M, Svergun D, **Bujnicki JM**, Le Grice S, Nowotny M. Structural analysis of monomeric retroviral reverse transcriptase in complex with an RNA/DNA hybrid. 2013. Nucleic Acids Res, in press
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- ***Nowotny M**, Gaidamakov SA, Ghirlando R, Cerritelli SM, Crouch RJ, Yang W. Structure of human RNase H1 complexed with an RNA/DNA hybrid: Insight into HIV Reverse Transcription. Mol Cell, 2007; 28:264-276
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Description of Current Research

Our laboratory focuses on structural and biochemical studies of nucleic acid enzymes using protein crystallography as a primary method. The key results obtained recently in our laboratory concern three proteins: RNases H2, reverse transcriptases, and UvrA.

1. Structural studies of bacterial RNases H2

RNases H are small nucleases that specifically hydrolyze RNA in RNA/DNA hybrids. They are divided into two types—RNases H1 and RNases H2—that have a similar structure of the catalytic core but different domain organization and biochemical properties. The most important feature of RNases H2, differentiating them from type 1 enzymes, is their ability to cleave single ribonucleotides embedded in the DNA. Such single ribonucleotides occur quite frequently in genomic DNA and most often result from misincorporation by DNA polymerases. They must be removed to maintain genomic stability, and RNase H2 is the only enzyme that can initiate this process by cleaving the phosphate linkage on the 5′ side of the ribonucleotide. The removal is completed by the second cut on the 3′ side of the RNA by FEN-1 endonuclease.

The mechanism of the specific cleavage of single ribonucleotides by RNase H2 was previously unknown. To elucidate this, we solved the crystal structures of *Thermotoga maritima* RNase H2 in complex with the nucleic acid substrate. The results showed that the nucleic acid is bound in a cleft between the *N*-terminal catalytic domain and *C*-terminal helical domain (cover figure). The key element that ensures the substrate specificity of the enzyme is the recognition mechanism for a (5′)RNA-DNA(3′) junction. The RNA residue of the junction forms a network of interactions between its 2′-OH and the backbone of three protein residues—two glycines and an arginine—that form an element we call the "GRG motif." The hydroxyl group of an absolutely conserved tyrosine residue from the *C*-terminal domain also forms a hydrogen bond with the 2′-OH group. This tyrosine also interacts with the second group of

the junction, forming a stacking interaction with its ribose ring. This interaction can be efficient only if a 2'-OH group is absent from the ring and therefore is selective for deoxyribonucleotide in the second position of the junction. The stacking interaction leads to a deformation of the nucleic acid, changing the conformation of the phosphodiester backbone of the RNA-DNA junction. Because of this deformation, the phosphate group of the junction can participate in the coordination of a metal ion at the active site. This mechanism ensures very stringent substrate specificity. Only when a correct substrate is present (e.g., an RNA-DNA junction) that can be properly deformed can the metal ion be coordinated at the active site and the reaction proceed.

The active site of RNase H2 is formed by four conserved carboxylate residues. In the wild-type structure solved in the presence of Ca^{2+} ions, we observed three ions at the active site. Two of the ions occupied positions very similar to the two catalytic metal ions in related enzymes, and we assume that RNase H2 uses a canonical two-metal ion mechanism. In this mechanism, one metal ion activates the attacking nucleophile, and the second ion stabilizes the transition state and reaction product.

The studies of RNases H2 were performed in collaboration with Dr. Robert Crouch (National Institutes of Health, USA).

2. Structural studies of reverse transcriptases

Reverse transcription involves the conversion of single-stranded RNA to double-stranded DNA and is essential for the proliferation of retrotransposons and retroviruses, such as human immunodeficiency virus (HIV-1). Reverse transcription is an intricate multi-step process catalyzed by very versatile enzymes called reverse transcriptases (RTs). These enzymes possess two activities: DNA polymerase synthesizes the new DNA, and RNase H degrades the RNA strand in the RNA/DNA intermediates of the reaction, thus removing the original genetic information.

Retroviral RTs can be divided into two groups, based on their architecture. Dimeric enzymes, such as HIV-1 RT, are very well characterized structurally and biochemically, but the mechanism of monomeric RTs is less well understood. To characterize the mechanism, we solved the crystal structure of monomeric RT from xenotropic murine leukemia virus-related virus (XMRV) in complex with an RNA/DNA substrate. The structure comprised the polymerase domain, but the RNase H was disordered and hence not visible. The structure revealed that the active site and substrate contacts around it were well conserved between monomeric and dimeric RTs. Further toward the RNase H domain, however, substrate binding was mediated by a different set of residues. Our structure also revealed the role of a "pin" structure that guided the trajectory of the template strand and was important for DNA polymerase processivity. We also explained the ability of XMRV RT to perform so-called strand displacement DNA synthesis, during which a nucleic acid that is hybridized with the template ahead of the polymerase active site is removed concurrently with polymerization.

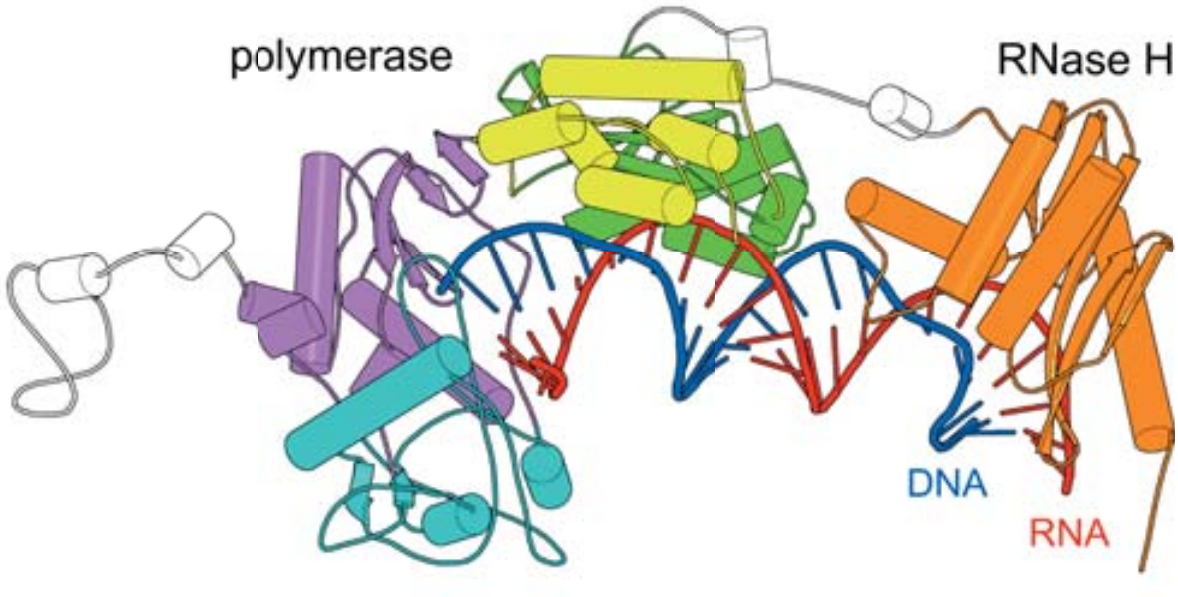
In collaboration with Janusz Bujnicki and Dmitri Svergun we used small-angle X-ray diffraction data and combined them with the crystal structures to model the full-length enzyme. These studies revealed that the RNase H domain was very mobile in the absence of nucleic acid and became organized on the substrate when it was present. Transient and infrequent interactions between the RNase H domain and substrate appear to be a universal feature of RTs. It likely allows the enzyme to regulate RNase H activity, which has to perform very precise cuts at several stages of reverse transcription. However, the mechanism of the regulation of the RNase H interaction with the substrate is very different for the HIV-1 enzyme. Unlike in monomeric XMRV RT, the RNase H domain in dimeric RTs is rigidly placed on the non-catalytic subunit, and the substrate has to be deformed to reach its active site. Reverse transcriptases, therefore, exhibit an intriguing variety of mechanisms to perform their function.

The studies of XMRV RT were performed in collaboration with Dr. Stuart Le Grice (National Institutes of Health, USA).

3. Structural and biochemical studies of UvrA DNA repair protein

DNA constantly undergoes detrimental chemical modifications (also called DNA damage) that occur spontaneously or are caused by physical and chemical factors. To maintain the genetic stability of the cell and protect the organism, these modifications need to be corrected. One of the primary pathways to achieve this is nucleotide excision repair (NER). The most important feature of NER is its ability to recognize a wide variety of DNA lesions of unrelated chemical structures. Different proteins are involved in NER in bacteria and eukaryotes, but the principle is the same. The site of damage is located, its presence is verified, and the DNA is incised on both sites of the lesion. The DNA fragment that contains the lesion is removed by a helicase, and the gap is filled by DNA polymerase. In bacteria, the first component of the pathway, which locates the lesion, is UvrA protein. It is a dimeric adenosine triphosphatase (ATPase) from the ATP-binding cassette (ABC) family. After the damage is found, the DNA is handed over to UvrB, which possesses weak helicase activity and verifies the presence of the lesion. UvrC nuclease executes the two cuts on the two sides of the modification.

The key unanswered question in NER is how its remarkably wide specificity is achieved. To elucidate this, we sought to solve the crystal structure(s) of a UvrA protein in complex with modified DNA. In our extensive crystallization trials, we used UvrA proteins from two bacterial species and DNA oligonucleotides that contained a single thymine residue with a fluorescein moiety attached through a flexible tether. We used DNA duplexes with a modified thymine residue in one of the DNA strands and duplexes that consisted of palindromic oligonucleotides that contain symmetrically placed modified thymines in both strands. We reasoned that the symmetry of such DNAs would reflect the two-fold symmetry of the UvrA dimer and hence promote crystallization. Indeed, we only obtained crystals with the palindromic oligonucleotides. We then used biochemical assays to verify that each of the strands of the palindromic substrates can be independently processed by the NER machinery that consisted of UvrA, UvrB, and UvrC. The crystals diffracted X-rays up to 2.9 Å resolution, and the structure was solved using molecular replacement. In the structure, the DNA is bound in a cleft that runs across the

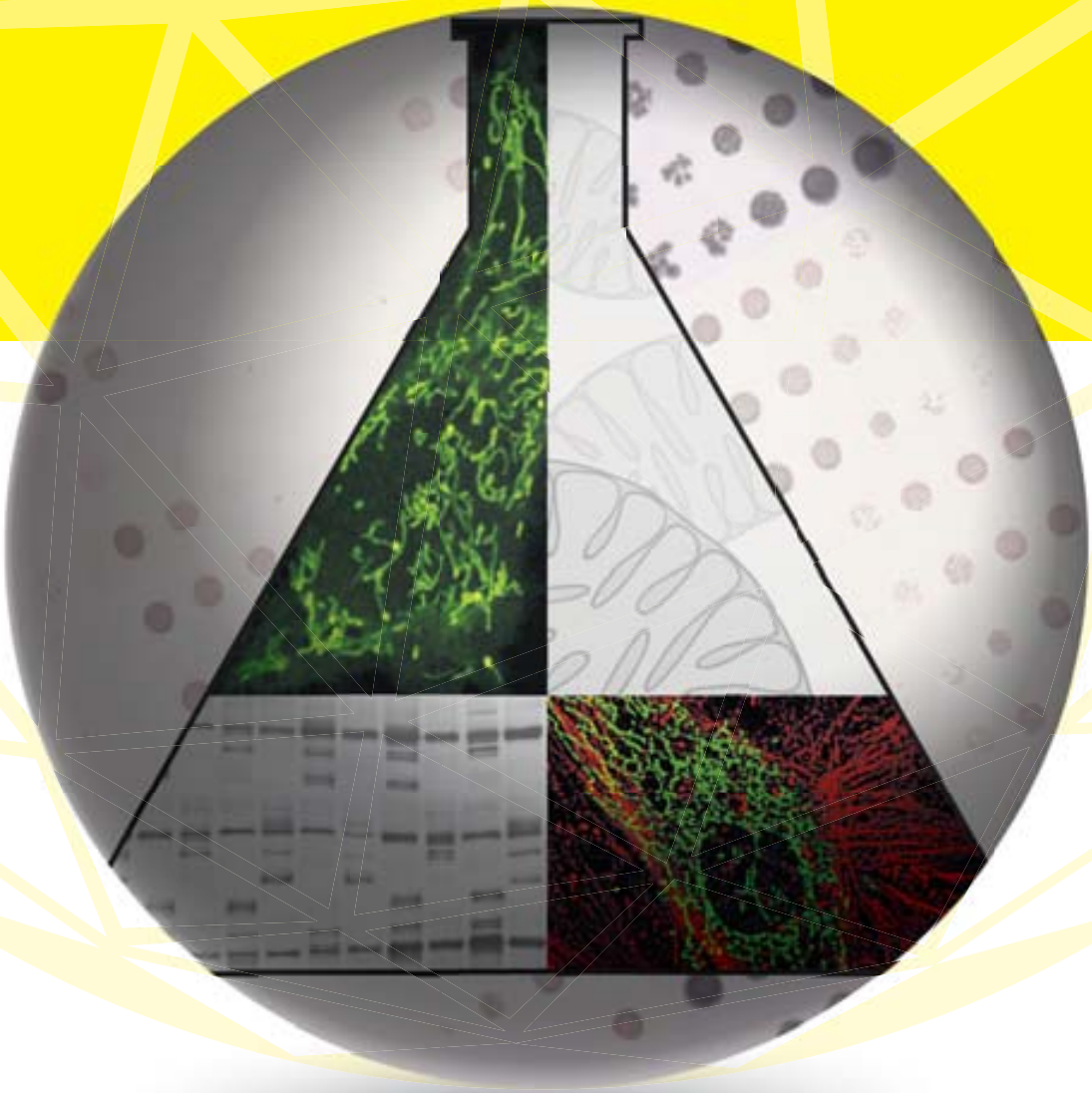


Model of full-length monomeric XMRV RT prepared based on crystal structures and small-angle X-ray scattering data. The DNA polymerase domain is shown in pink, cyan, and yellow. Connection and RNase H domains is green and orange. The RNA/DNA substrate is shown red and blue.

UvrA dimer. The interactions between the protein and nucleic acid are formed almost exclusively with the terminal regions of the DNA duplex. We identified a conserved, positively charged patch on the surface of the protein that forms extensive contacts with the DNA backbone.

The key to DNA damage recognition by UvrA is the conformation of the DNA. The duplex is bent by approximately 15 degrees, stretched in the middle, and unwound. Only this deformed conformation is complementary with the protein surface. The DNA deformations we observe are also often seen in various modified DNAs in free, unbound form. Unwinding is a particularly common feature of many damaged DNAs. Therefore, we proposed that UvrA uses an indirect readout mechanism to detect the presence of the damage. The protein senses the deformations of the DNA caused by the lesion. At the same time, it may also adjust those deformations so that the duplex fits to the protein surface. Modified DNA duplexes are known to be more flexible and easier to deform. UvrA probes the conformation of the DNA symmetrically on both sides of the lesion without directly interacting with the site of modification itself. Its dimeric structure is ideally suited for this purpose, but the symmetrical damage detection does not provide information about which strand is damaged and needs to be incised. This is most likely the role of the UvrB protein, which is recruited to the DNA after UvrA finds the damage site.

The mechanism of indirect readout we described is unique. Eukaryotic NER proteins, for which crystal structures are available, such as UV-DDB and XPC/HR25 complexes, form specific contacts with the site of lesion and use base flipping to probe the strength of the base pair hydrogen bonds to detect the damage.



Graphics by Agata Trojanowska

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Education and Degrees

2008	DSc Habil, Institute of Biochemistry and Biophysics, Warsaw, Poland
2000	PhD in Biochemistry, Institute of Biochemistry and Biophysics, Warsaw, Poland
1993	MSc in Molecular Biology, University of Warsaw
1988-1993	Biology, University of Warsaw, Poland

Awards

2010	EMBO Installation Grant
2009	Welcome Programme, Foundation for Polish Science
2008	Eugen-Graetz Prize for Research, University of Freiburg, Germany
2001-2003	Long-term FEBS fellowship
2001	Award for PhD thesis, Institute of Biochemistry and Biophysics, Warsaw, Poland
1997	Grant for Young Scientists, Polish State Committee for Scientific Research
1996	Short-term FEBS fellowship

Research experience and Appointments

2009-Present	Professor and Head of Laboratory of Mitochondrial Biogenesis, International Institute of Molecular and Cell Biology, Warsaw, Poland
2008-2009	Associate Member of Excellence Cluster BIOSS, Centre for Biological Signalling Studies, University of Freiburg, Germany
2007-2009	Member of the Board, Collaborative Research Centre (SFB 746)
2007-2010	Project Leader in Collaborative Research Centre (SFB 746)
2004-2009	Group Leader (German equivalent of Assistant Professor), Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany
2001-2004	Postdoctoral Fellow, Laboratory of Prof. Nikolaus Pfanner, University of Freiburg, Germany
1999	Visiting Scientist, Laboratory of Prof. Sabine Rospert, Max Planck Research Unit, Halle, Germany
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1994-2000	Doctoral research with Prof. Magdalena Boguta, Institute of Biochemistry and Biophysics, Warsaw, Poland

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2012

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(Publications until 2009 have no IIMCB affiliation)

Current Research

Mitochondria play an important role in metabolism and regulatory processes in the cell. Thus, the formation of mitochondria is essential for cellular function in the entire eukaryotic kingdom, from unicellular organisms to mammals. Mitochondria comprise 1000-1500 cellular proteins that are synthesized outside the mitochondria in the cytosol and must be imported into mitochondria (Fig. 1). The biogenesis

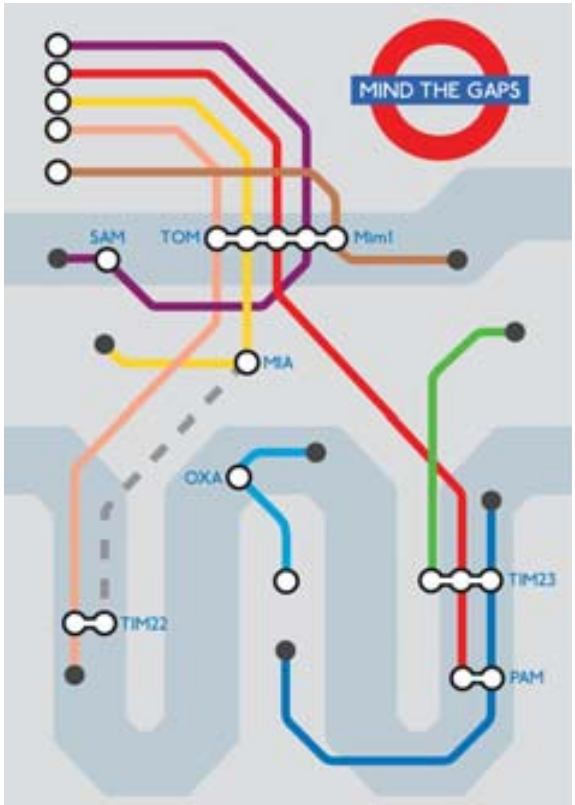


Fig. 1. Schematic representation of the import and sorting pathways that govern the distribution of proteins in mitochondria. Graphics by Agata Trojanowska.

of mitochondria relies on the efficient import, sorting, and maturation of proteins governed by conserved protein translocases and other complex machineries. In the course of earlier work at the University of Freiburg, we made a surprising discovery that contradicted the dogma on the absence of disulfide bonds in reducing cellular compartments, such as mitochondria. We identified and characterized a novel mitochondrial intermembrane space assembly (MIA) pathway that utilizes the transfer of disulfide bonds and is dedicated to the import and biogenesis of intermembrane space proteins that lack a classical mitochondrial leader sequence (Fig. 2).

Supported by a Welcome Grant from the Foundation for Polish Science, an EMBO Installation Grant, and grants from the Ministry of Science and Higher Education and National Science Centre, the group seeks to understand the complex and dynamic processes involved in the formation of functional mitochondria, the maintenance of mitochondrial protein homeostasis, and their failure that results in pathology. Our major interests are related to redox-dependent processes involved in mitochondrial protein biogenesis. We concentrate on the following issues:

- Redox-related biogenesis events of mitochondrial proteins in yeast and higher eukaryotes.
- Impact of the MIA pathway on mitochondrial and cellular protein homeostasis.
- Biological consequences of oxidative protein biogenesis failure.

Redox-based protein biogenesis events in the intermembrane space of mitochondria

Our research aims to understand the biogenesis of proteins localized in the intermembrane space of mitochondria. To be entrapped in the intermembrane space of mitochondria, proteins utilize catalyzed thiol-disulfide exchange driven by mitochondrial intermembrane space assembly (MIA) machinery. One interesting mechanistic aspect under debate is the mode of cooperation between Mia40 and Erv1, two major components of the MIA pathway (Fig. 2). In contrast to the well-established view that Mia40 interacts with either substrate proteins to facilitate their oxidative folding or Erv1 for Mia40 reoxidation, we provided compelling evidence in organelle and in vivo that the oxidation of intermembrane space substrate proteins involves the simultaneous association of Mia40 and Erv1 to maintain the productivity of oxidative biogenesis (Böttinger et al., 2012). These findings led us to propose that the oxidative folding of intermembrane space proteins governed by MIA is a spatially and temporally coordinated chain of events (for review, see Stojanovski et al., 2012).

We are interested in studies performed in higher eukaryotes. The components of the MIA pathway that are similar to other mitochondrial protein translocases are conserved, but they have remained only poorly understood. Our currently completed intermediate stage in reaching the goal of understanding redox-driven protein biogenesis in human cells involved reconstituting the human MIA pathway in yeast. We exchanged the essential yeast counterparts for the human proteins MIA40 and ALR (the sulfhydryl oxidase, yeast Erv1 homolog) and established the mechanistic principles of the human MIA pathway. Importantly, with the use of our "humanized" yeast, we addressed the exact molecular defect caused by the mutant variant of ALR. We found a new role for ALR in the biogenesis of human MIA40 and demonstrated that the defective accumulation of human MIA40 in mitochondria underlies the pathology of the disease variant of ALR (Sztolsztener et al., 2012).

Regulation of redox-driven protein biogenesis

We previously demonstrated (Milenkovic et al., 2007; Milenkovic et al., 2009) that mitochondrial precursor proteins are specifically recognized by Mia40, the major component of the MIA pathway, after they pass a main entry gate into mitochondria formed by the TOM complex (Fig. 2). Thus, Mia40 acts a receptor for intermembrane space proteins. Subsequent to the recognition event, Mia40 engages precursors via the formation of intermolecular disulfide bridge. However, Mia40 is located in the mitochondrial inner membrane, and this membrane is folded in structures called cristae. Our data led us to conclude that Mia40 is located in close proximity to the TOM complex. In the search for factors that determine the localization of Mia40, we performed a comprehensive study of protein interactions. We identified a new interaction partner of Mia40, Fcj1 (Formation of Crista

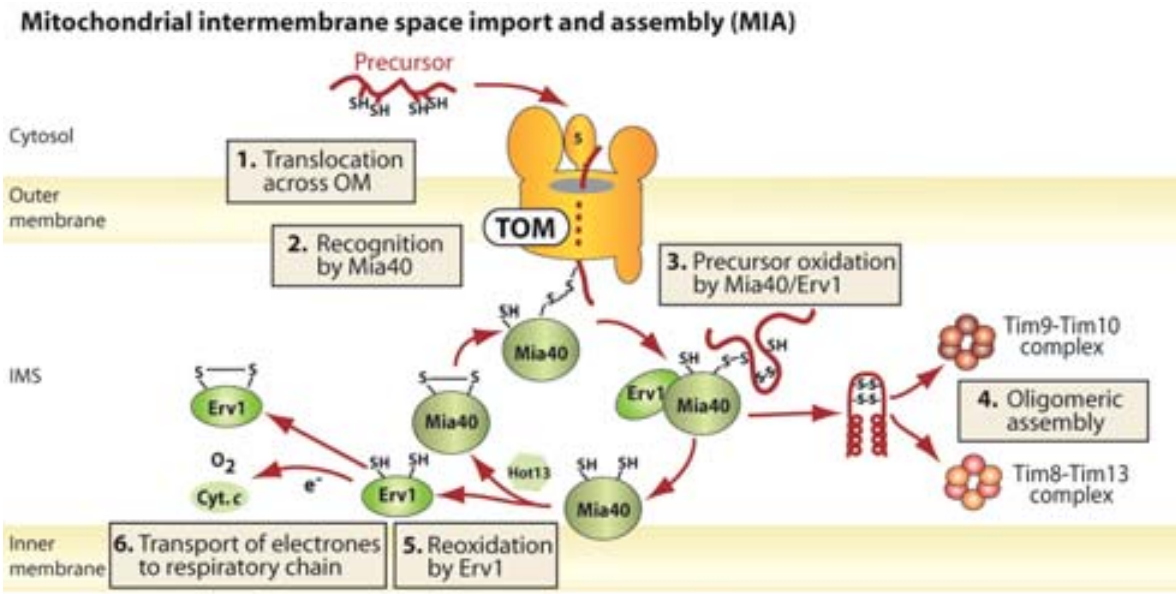


Fig. 2. Intermembrane space precursor proteins are posttranslationally transferred to the mitochondria via TOM. The possibility exists that protein synthesis is coupled to protein transport. After arriving on the trans side of the TOM complex, intermembrane space proteins enter the MIA pathway, driving their import completion and maturation by catalyzing disulfide bond formation and folding.

Junctions; mitofilin in higher eukaryotes), and demonstrated that Fcj1 interacts with the TOM complex. Thus, Fcj1 is a regulatory factor that spatially organizes the biogenesis of mitochondria by positioning Mia40 in close proximity to the TOM complex (Fig. 3). Moreover, consistent with this general function of Fcj1 in the spatial organization of mitochondria, we also characterized a large complex formed by Fcj1 that we named MINOS for its critical role in mitochondrial inner membrane organization (von der Malsburg et al., 2011). We are continuing to study the relationship between the MIA pathway and Fcj1 and its role in membrane organization.

Redox-driven protein biogenesis events beyond the intermembrane space of mitochondria

In the search for non-canonical functions of MIA, we investigated inner mitochondrial membrane proteins. Surprisingly, a multispanning membrane protein responsible for the transport of mitochondrial inner membrane proteins, Tim22, was found in the oxidized state in mitochondria. We demonstrated that Tim22 transiently interacts with Mia40 via disulfide bonds. Furthermore, Tim22 can also interact with Mia40 via non-covalent hydrophobic interactions. In conclusion, Mia40 serves not only as an oxidoreductase but also as a translocase that assists inner membrane proteins in their passage through the intermembrane space and membrane integration (Wrobel et al., 2013, manuscript in press). This finding extends the function of the MIA pathway beyond the oxidative folding of intermembrane space proteins. The mechanism and precise role of cysteine residues in Tim22 and selected other membrane proteins in membrane insertion remain core subjects of our current research.

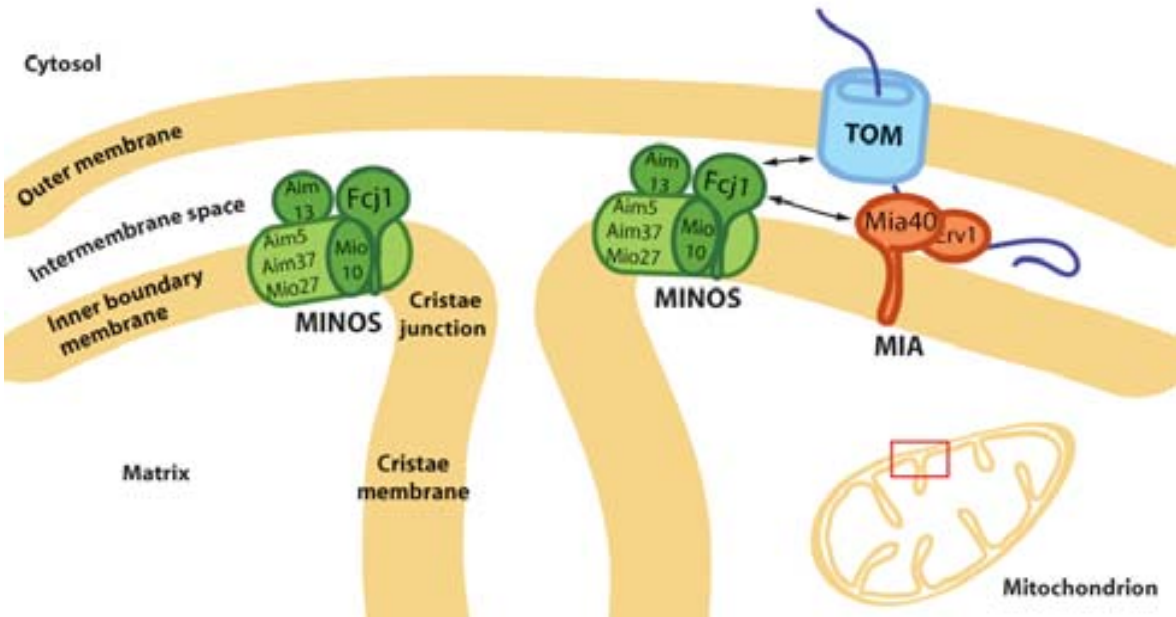
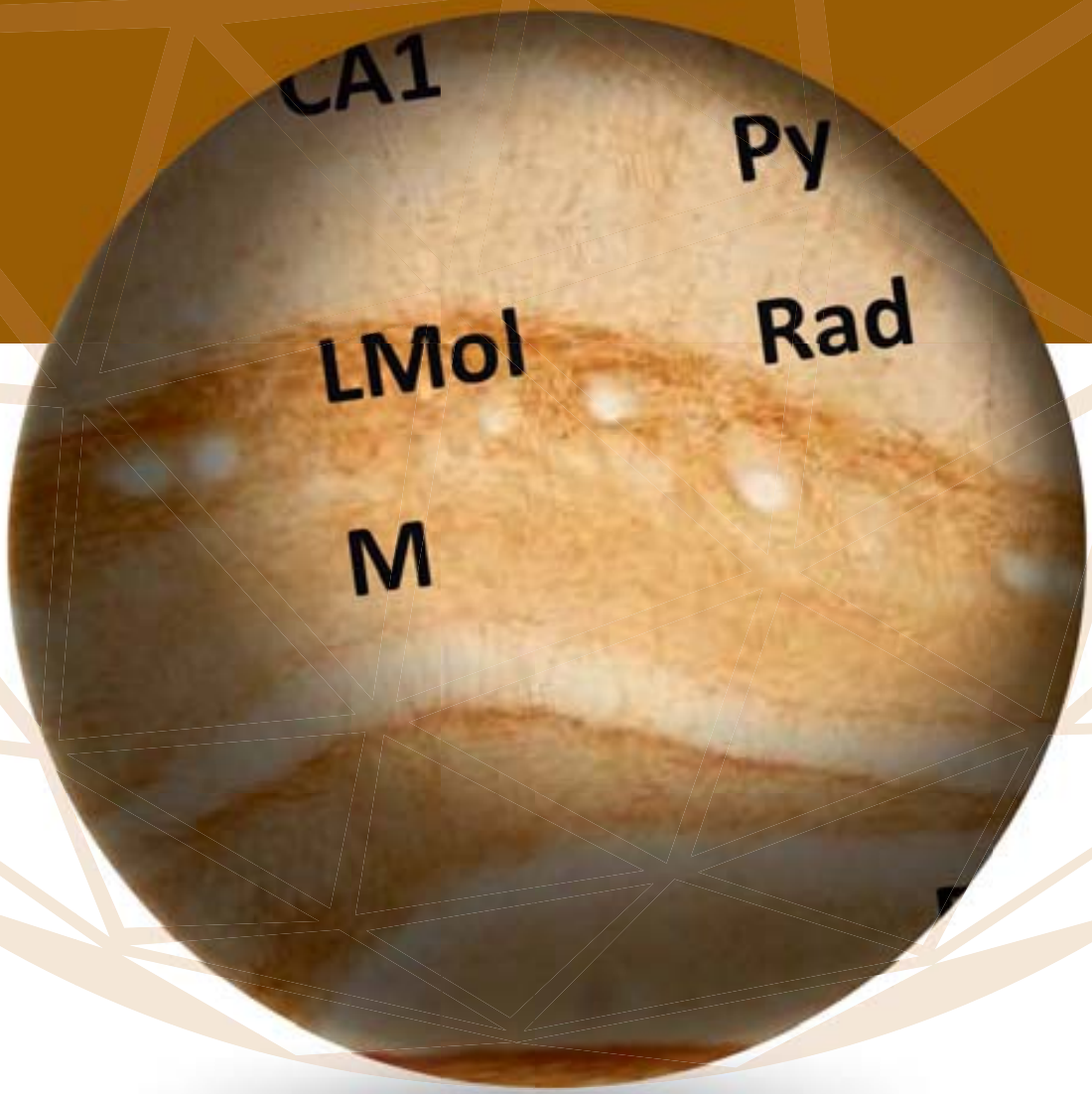


Fig. 3. Fcj1 is a component of the MINOS complex that is critical to the maintenance of the unique architecture of the inner mitochondrial membrane folded in cristae. It is also involved in the spatial organization of mitochondrial protein biogenesis by positioning Mia40, the receptor for intermembrane space proteins, in direct contact with precursors that arise on the trans side of the TOM complex.



DAB-staining of mice brain slices visualizing major component of *Myelin Sheet* – Myelin Basic Protein (MBP) in Hippocampus and Cortex (Author Łukasz Szewczyk).

Laboratory of **Neurodegeneration**

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Associate Professor, Vice Head:
Urszula Wojda, PhD, DSc Habil

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Marta Wiśniewska, PhD

Senior Postdoctoral Fellow:
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Postdoctoral Fellows:
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FishMed Technical Assistant:
Michał Bazała, MSc (from Dec. 2012)

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Emilia Białopiotrowicz, MSc (until September 2012); Kinga Gazda, MSc in Engineering (since September 2012); Anna Jaworska, MSc; Kamil Koper, MSc (since November 2012); Andrzej Nagalski, MSc; Katarzyna Potrzebowska (Dębowska), MSc (until September 2012); Katarzyna Sawicka, MSc (since October 2012); Łukasz Szewczyk, MSc; Aleksandra Szybińska, MSc

MSc Students:
Nikola Brożko (until June 2012)

Technician:
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- Emilia Białopiotrowicz, postdoctoral research fellow, Laboratory of Experimental Hematology, Institute of Hematology and Transfusion Medicine, Warsaw
- Magdalena Błażejczyk, postdoctoral research fellow, Laboratory of Molecular and Cellular Neurobiology, IIMCB
- Łukasz Bojarski, research group leader, New Therapies of Neurological Diseases, Celon Pharma (www.celonresearch.com)
- Danuta Korona, student at Deutsches Krebsforschungszentrum (DKFZ), Studying Cancer Biology at University of Heidelberg, Germany
- Bożena Kuźniewska, PhD student in the laboratory of Prof. Leszek Kaczmarek, Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Warsaw
- Wojciech Michowski, postdoctoral research fellow, Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA
- Katarzyna Misztal, postdoctoral research fellow, Laboratory of Structural Biology, IIMCB
- Katarzyna Potrzebowska (Dębowska), postdoctoral research fellow, Leukocyte Migration Group, Immunology Section, Department of Experimental Medical Sciences, Lund University, Lund, Sweden
- Adam Sobczak, postdoctoral research fellow, Institute of Biochemistry and Biophysics Polish Academy of Sciences, and Bio&Technology Innovations Platform of BioCentrum Ochota (BioTech-IP)

Head of Laboratory of Neurodegeneration

Jacek Kuźnicki, PhD, Professor

Degrees:

1993	Professor, nomination by the President of the Republic of Poland
1987	DSc Habil, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1980	PhD in Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1976	MSc in Biochemistry, Warsaw University, Poland

Postdoctoral Training

1981-1984	Visiting Fellow, Laboratory of Cell Biology (Head: E.D. Korn), National Institutes of Health, Bethesda, Maryland, USA

Professional Employment:

2002-Present	Director of the Institute and Head of the Laboratory of Neurodegeneration, IIMCB
2000-2001	Director, Centre of Excellence for Studies on Mechanisms of Neurodegeneration Phare Sci-Tech II, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1999-2001	Acting Director, IIMCB; Organizer and Director, Centenarian Program
1996-2002	Head, Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1992-1995	Visiting Professor, National Institute of Mental Health, Laboratory of Clinical Science, Bethesda, Maryland, USA
1991-1992	Deputy Director (Scientific Director), Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1986-1992	Associate Professor and Head, Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1984-1985	Research Associate, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1981-1984	Visiting Fellow, National Institutes of Health, Laboratory of Cell Biology, Bethesda, Maryland, USA
1980-1981	Postdoctoral Fellow, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
1976-1980	PhD Student, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Membership in Scientific Societies, Organizations, and Panels:

Jul 1, 2012 – Dec 31, 2012	President of the Science Policy Committee at the Ministry of Science and Higher Education (rotating presidency); member since 2011
2012-Present	Board Member of Marceli Nencki's Foundation to Support the Biological Sciences
2011-Present	Member, International Expert Council of the Research and Education Center, State Key Laboratory of Molecular and Cellular Biology (SKL) in Ukraine
Oct-Nov 2011	Chairman of the Commission for the Assessment of Property and Legal and Organizational Joined PAN Scientific Units (units operating under the name of the Department of Antarctic Biology Polish Academy of Sciences and Institute of Biochemistry and Biophysics)
2011-Present	Member, BIO-IMAGINE Steering Committee, 7th Framework Program at the Nencki Institute of Experimental Biology
2011-Present	Member, Science Policy Committee, Ministry of Science and Higher Education
Jul 1, 2010 – Dec 31, 2010	President, Consortium Biocentrum Ochota (rotating presidency)
2010-Present	Member, Society for Neuroscience
2008-2010	Head, Scientific and Organizing Committees, 11th Meeting of the European Calcium Society
2009-Present	Member, Polish Alzheimer's Society
2008-Present	Board Member, European Calcium Society
2006-Present	Member, Health Research Advisory Group, 7th Framework Program European Commission
2004-Present	Member, Polish Academy of Sciences
2003-Present	Member, American Society for Biochemistry and Molecular Biology
2002-Present	Head, Advisory Board, Centre for Innovative Bioscience Education
1991-Present	Member, Polish Neuroscience Society
1991-2009	Member, Polish Society for the Advancement of Science and Arts
1996-1999, 2000-2002	Vice-President, Polish Biotechnology Committee
1990-2002	Member, Polish Biotechnology Committee
1989-1992	Co-Editor, *Advances in Biochemistry* (published in Polish)
1989-1991	General Secretary, Polish Biochemical Society
1977-Present	Member, Polish Biochemical Society

Honors, Prizes, and Awards:

2011 Konorski Award for the best Polish research work in neurobiology (awarded by the Polish Neuroscience Society and Committee on Neurobiology of PAN)

2008 Officer's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)

2004-2008 Professorial Subsidy Program Award, Foundation for Polish Science

2003 Prime Minister Award for Scientific Achievement

2001 Award from the Division of Biological Sciences, Polish Academy of Sciences (for work on calcium binding proteins)

1998 Knight's Cross of the Order of Polonia Restituta (awarded by the President of the Republic of Poland)

1987 Polish Anatomical Society Award for article on calcium binding proteins (*Advances in Cell Biology*)

1986 Skarżyński Award, Polish Biochemical Society (for best review article in *Advances in Biochemistry*)

1977 Parnas Award, Polish Biochemical Society (for publishing the best paper in biochemical research)

1977 Mozołowski Award, Polish Biochemical Society (for outstanding young Polish biochemists)

1976 MSc, Magna cum laude, University of Warsaw, Poland

Selected publications

- **Jaworska A**, Dzbek J, Styczynska M, **Kuznicki J**. Analysis of calcium homeostasis in fresh lymphocytes from patients with sporadic Alzheimer's disease or mild cognitive impairment. BBA Mol Cell Res, 2013 Jan 24. doi:pii:S0167-4889(13)00025-6. 10.1016/j.bbamcr.2013.01.012. [Epub ahead of print]
- **Wojda U, Kuznicki J**. Alzheimer's Disease Modeling: Ups, Downs, and Perspectives for Human Induced Pluripotent Stem Cells. J Alzheimers Dis, 2013 Jan 11. [Epub ahead of print]. DOI 10.3233/JAD-121984
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- **Wisniewska MB**. Physiological Role of β-Catenin/TCF Signaling in Neurons of the Adult Brain. Neurochem Res. 2013 Feb 2. [Epub ahead of print]. DOI 10.1007/s11064-013-0980-9
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* no IIMCB affiliation

Current Projects

We are interested in the molecular mechanisms involved in neurodegeneration and memory formation, with a special emphasis on the role of calcium homeostasis and signaling. These processes are being studied at the genomic, proteomic, and cellular levels and using zebrafish, and mice as model organisms. Our major projects focus on the following:

1. Calcium homeostasis and calcium signaling

1.1. Role of STIM proteins in store-operated calcium entry in neurons

1.2. Functional and physical interaction between Alzheimer's disease proteins and store-operated calcium entry machinery

1.3. Function of calmyrins in neuronal physiology and pathology

1.4. Dysregulation of calcium homeostasis in Alzheimer's disease

2. Search for biomarkers and potential therapeutic targets in lymphocytes from Alzheimer's disease patients

2.1. Calcium measurements

2.2. Analyses of the cell cycle and apoptosis

3. Role and regulation of β-catenin and transcription factors LEF1/TCF in mature neurons

4. Role of epigenetic mechanisms in regulation of calcium-related genes involved in neurodegenerative diseases

5. Transgenic mice with dysregulated calcium homeostasis in neurons as a model of aged-induced neurodegeneration

1. Calcium homeostasis and calcium signaling

1.1. Role of STIM proteins in store-operated calcium entry in neurons (Joanna Gruszczyńska-Biegała)

The calcium sensors STIM1 and STIM2, located in the endoplasmic reticulum (ER), and calcium channel-forming protein ORAI1 are involved in store-operated calcium entry (SOCE). The process relies on extracellular calcium influx through plasma membrane channels. In non-excitable cells, the STIM interaction with ORAI1 is a crucial element of SOCE, but its mechanism remains unclear in neurons. We previously showed that STIM1 is likely involved in thapsigargin-induced SOCE, whereas STIM2 is mostly active after the ethylene glycol tetraacetic acid-driven depletion of extracellular calcium (Gruszczynska-Biegala et al., *PLoS One*, 2011). The depletion of calcium from the ER by thansigargin increased the number of puncta-like colocalization of YFP-STIM1 and ORAI1 but not YFP-STIM2 and ORAI1. In contrast, a reduction of extracellular calcium levels triggered puncta formation for both YFP-STIM1/ORAI1 and YFP-STIM2/ORAI1. As a next step, we focused on detecting complexes that contain endogenous STIM2 and ORAI1. Using a proximity ligation assay (PLA), we were able to visualize fluorescent dots that represent the site where two antibodies are bound: one against ORAI1 and another against STIM2. These dots identified the complexes between STIM2 and ORAI1. To confirm that the observed PLA dots represented authentic STIM2-ORAI1 complexes, we used different pairs of anti-STIM2 and anti-ORAI1 antibodies. The number of these complexes increased when intracellular and subsequently ER calcium concentrations decreased under the influence of BAPTA-AM or medium without calcium ions. These results were confirmed by co-immunoprecipitation of endogenous STIM2 and ORAI1 proteins. We also showed a strong correlation between the number of endogenous STIM2-ORAI1 complexes and calcium responses studied in the same neuronal cell. Our results indicated that STIM2 responds to changes in intracellular calcium levels and the small decrease in calcium levels in the ER in rat cortical neurons by interacting with ORAI1.

1.2. Functional and physical interaction between Alzheimer's disease proteins and store-operated calcium entry machinery (Tomasz Węgierski, Kinga Gazda)

The genetic manipulation of proteins linked to Alzheimer's disease (AD) results in disturbances in cellular calcium homeostasis. Specifically, alterations in the receptor-induced release of calcium from the ER and SOCE have been described. These observations support the calcium hypothesis of the development of AD, but the precise mechanisms that underlie the dysregulation of calcium homeostasis in AD models are unclear. We aim to elucidate these mechanisms, particularly whether AD proteins exert a direct regulatory effect on key players of calcium homeostasis, such as the SOCE complex. Using a split-ubiquitin system (i.e., a yeast genetic system) to search for interacting partners, we found a physical interaction between SOCE machinery and proteins crucially involved in the development of neurodegeneration. The interaction was confirmed using independent methodology, such as co-immunoprecipitation and co-immunolocalization assays. The functional relevance of this finding is being studied using overexpression systems and ablation of gene expression by RNA interference in various cell lines. We analyze the regulation of SOCE complexes using PLAs and calcium measurements with the help of the calcium indicator fura-2.

1.3. Function of calmyrins in neuronal physiology and pathology (Katarzyna Dębowska; supervisor: Urszula Wojda)

Neuronal Ca^{2+} signaling regulates multiple cellular functions. Therefore, disturbances in Ca^{2+} signaling pathways can result in neuronal pathologies. We study the neuronal function of a novel family of Ca^{2+} signaling proteins called calmyrins (CaMy; also known as KIP or CIB proteins). We characterized the biochemical properties, localization, and protein ligands of CaMy1 and CaMy2 in the brain and showed that CaMy1 is involved in AD (*BBA-Mol Cell Res*, 2011; *Arch Biochem Biophys*, 2009; *Calcium Binding Proteins*, 2008; *BBA-Mol Mech Diseases*, 2006; *Neuropathol Appl Neurobiol*, 2005; *Acta Biochim Pol*, 2005). Moreover, we identified the SCG10 protein stathmin2 as a novel CaMy1 ligand in the human brain. SCG10 is a microtubule-destabilizing factor that plays a role in neuronal growth during brain development. Our study demonstrated that CaMy1, via SCG10, coupled Ca^{2+} signals with the dynamics of microtubules during neuronal outgrowth in the developing brain (*BBA-Mol Cell Res*, 2011). More recently, we searched for the neuronal localization and function of another CaMy family member, CaMy2. We found that CaMy2 was preferentially expressed in neurons in the hippocampus and cortex. Endogenous CaMy2 was present in neurites and the Golgi apparatus and was found in the membranous fraction. Our search for CaMy2 protein ligands in neurons using affinity chromatography, mass spectrometry, and co-immunoprecipitation approaches revealed that CaMy2 interacted with key proteins involved in vesicular trafficking *in vitro* and *in vivo*, consistent with subcellular localization in neurons. Moreover, using RNA interference in

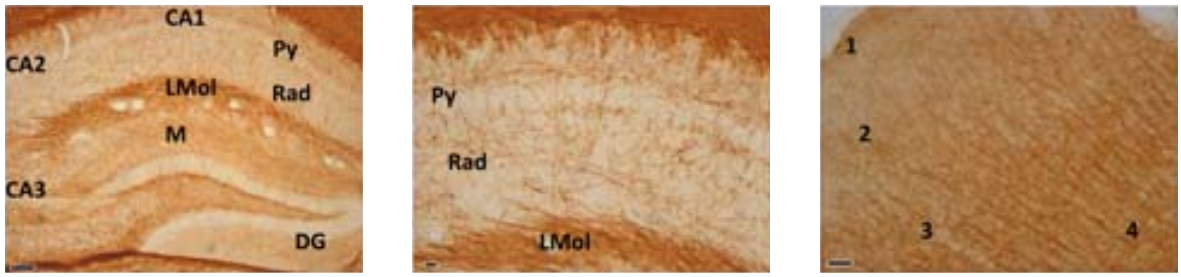

Fig. 1. DAB-staining of mice brain slices visualizing major component of *Myelin Sheet* – Myelin Basic Protein (MBP) in Hippocampus and Cortex (Author Łukasz Szewczyk).

primary hippocampal cultures, we demonstrated that CaMy2 affected the localization of early endosomes and endocytosis of neuronal surface receptors.

1.4. Calcium homeostasis in Alzheimer's disease (Aleksandra Szybińska, Anna Jaworska, and Tomasz Węgierski; collaboration: Honarnejad Kamran and Jochen Herms, Munich Center for Neurosciences)

Calcium dyshomeostasis is an early event in the pathogenesis of AD that precedes other disease symptoms and can affect many cellular processes. Drugs with the ability to restore calcium homeostasis to values observed in healthy control cells could be applied as therapeutics in AD. In collaboration with Prof. Jochen Herms, we screened approximately 20,000 chemical compounds to determine their ability to influence intracellular calcium concentrations. The screen revealed over 300 compounds that decreased calcium levels. To address their putative mechanism of action, almost 160 of the best compounds were chosen for an enzyme-linked immunosorbent assay (ELISA) for γ-secretase activity, whose gain of function is believed to be a major factor in familial AD pathology. Using ELISA, we measured β-amyloid 1-42 levels in HEK 293 cells that overexpressed the wildtype or mutated presenilin 1 gene. Only a few compounds decreased β-amyloid 1-42 to control levels; thus, the majority of the compounds that influenced calcium signaling did not affect γ-secretase activity.

2. Search for biomarkers and potential therapeutic targets in lymphocytes from Alzheimer's disease patients

Some molecular changes in AD can be observed not only in neurons but also in peripheral cells, such as lymphocytes. Because of difficulties studying dynamic processes in postmortem material, such peripheral cells have been used as a model to study the molecular mechanisms of AD. Additionally, human lymphocytes have potential diagnostic value. In our studies, we use B-lymphocytes from AD patients.

2.1. Calcium measurements (Anna Jaworska)

Many studies have shown that disturbed cellular calcium homeostasis is one of the key features of AD. Calcium changes can be observed not only in neurons but also in peripheral cells, such as skin fibroblasts and lymphocytes. Lymphocytes, in contrast to other cell types, can be easily obtained and therefore have great diagnostic potential. Disturbed calcium handling was found by many research groups in immortalized human B-lymphocytes derived from patients with an inherited form of AD (i.e., familial AD), but observations of similar changes observed in cells derived from patients with the sporadic form of AD (SAD) are very limited. Mild cognitive impairment (MCI) is found to be a transitional stage between normal aging and dementia. It is often observed in individuals who develop AD later in life and therefore may be considered a risk factor for AD. To explore calcium homeostasis during the early stages of SAD and MCI, we investigated SOCE and inositol triphosphate receptor (IP_3R)-mediated calcium release into the cytoplasm in unmodified B-lymphocytes from MCI subjects and SAD patients and compared them with non-demented subjects (NDS). Calcium levels in the endoplasmic reticulum were significantly similar in all three groups. However, we found that SAD and MCI cells were more prone to IP_3R activation than NDS cells. Mild cognitive impairment cells exhibited an enhanced magnitude of calcium influx during SOCE, and MCI cells but not SAD cells were characterized by higher basal cellular calcium levels than NDS cells. In summary, perturbed calcium homeostasis was observed in peripheral cells from MCI and SAD patients, supporting the hypothesis that SAD is a systemic disease, and MCI is a risk factor for AD. Thus, lymphocytes obtained from MCI subjects may be promising in the early diagnosis of individuals who will eventually develop SAD (*BBA MCR*, 2013).

2.2. Analyses of the cell cycle and apoptosis (Emilia Białopiotrowicz and Katarzyna Sawicka; supervisor: Urszula Wojda)

According to the so-called cell cycle (CC) hypothesis, an important factor that contributes to the pathogenesis of AD is the failure to regulate the G1/S phases of the cell cycle and CC reentry in differentiated, postmitotic neurons. Recently, we and others detected CC alterations in lymphocytes from SAD patients (Bialopiotrowicz et al., *Neurobiol Aging*, 2011). Our data showed that SAD involves a prolongation of the G1 phase driven by the p21 pathway, which is not activated in FAD cells. Thus, the mechanism of SAD is different from FAD. Moreover, disturbances of the CC in lymphocytes appear to have diagnostic value. Furthermore, we analyzed the effects of nine different PS1 mutations on CC regulation and Aβ production in immortalized lymphocytes from FAD patients and stably transfected human embryonic kidney cells. PS1 sustains the active site of γ-secretase, a membranous protein complex that cleaves transmembrane amyloid protein precursor (APP) to generate Aβ40 and Aβ42 peptides that in turn exert toxic effects in neurons. Mutations in PS1 that cause FAD increase the γ-secretase-mediated release of Aβ from APP. We found that both CC regulation and Aβ production differentiated PS1 mutations and that CC PS1 activity was mediated by p53/p21 signaling but not γ-secretase activity. The identified CC dysregulation linked with increased p53 and p21 protein levels distinguished the highly pathogenic PS1 P117R mutation and may contribute to the specific severity of the clinical progression of FAD associated with the mutation in the PS1 117 site (Fig 2). These findings suggest that impairment in the lymphocyte CC might play a pathogenic role in AD and may be relevant to the development of new diagnostic approaches and personalized therapeutic strategies (Bialopiotrowicz et al., *J Alzheimers Dis*, 2012).

3. Role and regulation of β-catenin and transcription factors LEF1/TCF in mature neurons (Katarzyna Misztal, Andrzej Nagalski, Łukasz Szewczyk, and Nikola Brożko; supervisor: Marta B. Wiśniewska)

β-catenin is a gene expression regulator in the canonical Wnt pathway that is involved in early brain patterning and neurogenesis. Growing evidence also implicates Wnt/β-catenin signaling in the proper functioning of the adult central nervous system. The aberrant regulation of β-catenin has been associated with psychotic and affective disorders (e.g., major depression, bipolar disorder, and schizophrenia) and neurodegenerative diseases (e.g., AD, Huntington's disease [HD], and Parkinson's disease). However, the physiological role of Wnt/β-catenin in the adult brain remains elusive. Pioneering

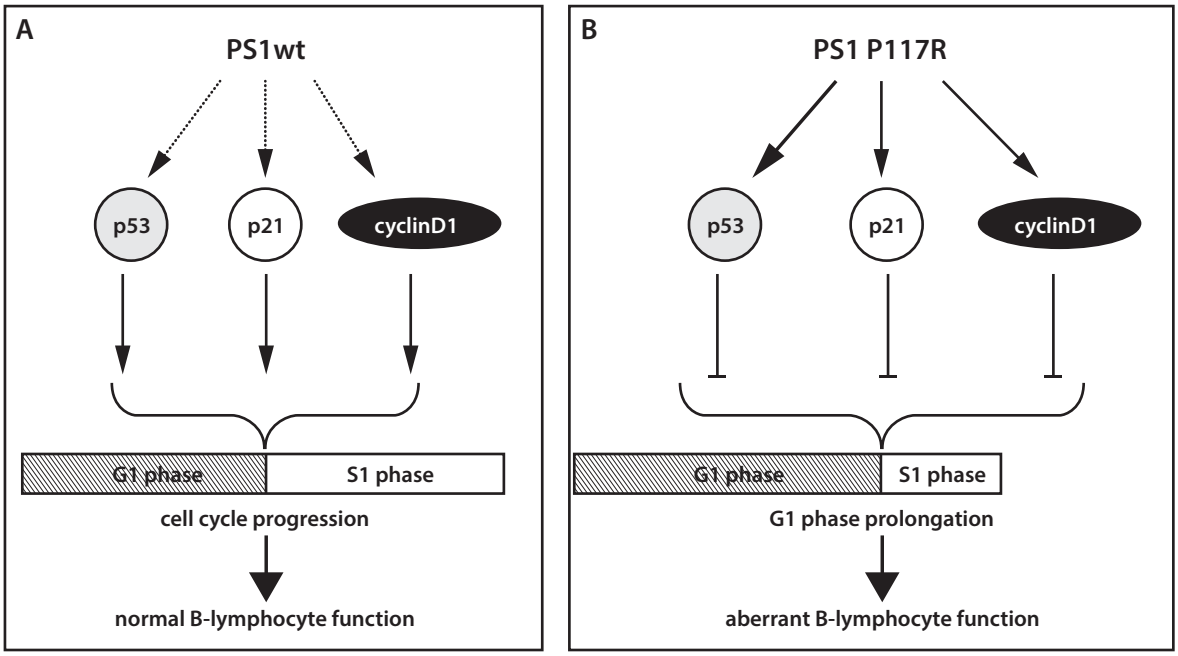

Fig. 2. Schematic representation of the mechanism leading to G1 phase prolongation and simultaneous S phase shortening in human lymphocytes harboring PS1 P117R mutation. In cells bearing PS1 P117R the levels of p53, p21 and cyclin D1 are high. Increase of cyclin D1 protein level might additionally positively stabilize p21 protein by protecting it from proteasomal degradation and thus potentiate p21 elevation. Increased levels of p53, p21 and cyclin D1 result in prolongation of G1 phase and shortening of S phase observed in PS1 P117R cells. The CC changes may affect functions of B lymphocytes and in this way may contribute to the specific severity of the clinical progression of FAD associated with the mutation in the PS1 117 site.

research by our group demonstrated that β-catenin is constitutively and specifically present in the nuclei of thalamic neurons, independent of Wnt signaling activation, and associated with low levels of the proteins involved in β-catenin degradation (i.e., APC, AXIN1, and GSK3β; Misztal et al., *J Biol Chem*, 2011). Moreover, we demonstrated that β-catenin, together with LEF/TCF transcription factors, regulated the transcription of the *Cacna1g* gene that encodes Cav3.1 voltage-gated calcium channels, contributing to electrical signal propagation in thalamic neurons (Wisniewska et al., *J Neurosci*, 2010). Recently, we identified new β-catenin target genes in thalamic neurons by combining bioinformatics and experimental approaches: *Gabra3* for the GABA receptor, *Calb2* for the Ca^{2+}-binding protein calretinin, and *Kcna6* for the voltage-gated potassium channel; Wisniewska et al., *BMC Genomics*, 2012). Two other genes, *Cacna2d2* and *Kcnh8*, appeared to be regulated by β-catenin, but the binding of β-catenin to the regulatory sequences of these genes could not be confirmed. We conclude that β-catenin in the thalamus regulates the expression of a novel group of genes that encode proteins involved in neuronal excitation. This implies that the transcriptional activity of β-catenin is necessary for the proper excitability of thalamic neurons, may influence activity in the thalamocortical circuit, and may contribute to thalamic pathologies. Additionally, we provided a comprehensive analysis of LEF1/TCF protein localization and the expression profile of their isoforms in cortical, thalamic, and midbrain regions in mice. The analysis of alternative splicing and promoter usage identified brain-specific TCF7L2 isoforms and revealed a developmentally coordinated transition in the composition of LEF1 and TCF7L2, suggesting that the role of these proteins in the adult brain might be different from their role in the embryonic brain.

4. Role of epigenetic mechanisms in the regulation of calcium-related genes involved in neurodegenerative diseases (Magdalena Czeredys; EraNet Russ project with Axel Methner, University of Mainz, Germany, and Elena Kaznacheyeva, St. Petersburg, Russia)

Calcium dyshomeostasis is an early event in the pathogenesis of neurodegenerative diseases. The literature suggests that epigenetic mechanisms, including DNA methylation and microRNA, can regulate gene expression in neurodegenerative diseases. Therefore, we focused on the expression of calcium-related genes in AD and HD. We hypothesized that some epigenetic changes might affect the expression of components of calcium homeostasis and signaling pathways, thereby initiating or propagating the neurodegenerative processes of AD and HD. To test this hypothesis, we analyzed mRNA levels in the brains of transgenic AD and HD mice using custom-made TaqMan Low Density Microarrays. Some genes whose expression was changed compared with control brains were further analyzed.

5. Transgenic mice with dysregulated calcium homeostasis in neurons as a model of age-induced neurodegeneration (Łukasz Majewski)

The vast majority of available animal models of AD are based on the β-amyloid/tau hypothesis. These mice overexpress one or more mutated proteins known to be responsible for the early onset of FAD. The FAD models, representing less than 5% of all human cases, appear to have little value for understanding the mechanisms of SAD. Our main research objective is to generate and characterize transgenic mouse models that have features characteristic of SAD. Transgenic mice with dysregulated Ca^{2+} homeostasis will be a suitable model for verifying the hypothesis that sustained increases in basal Ca^{2+} levels might be one of the early changes that lead to neurodegeneration.

Core Facility

Head: **Alicja Żylicz**, PhD, Professor

Senior Staff Scientists:
Krzysztof Skowronek, PhD
Roman Szczepanowski, PhD
Tomasz Węgierski, PhD

Radiation Safety Officer:
Piotr Brągoszewski, PhD

The Core Facility Laboratory was created in October 2011 with the goal of supporting innovative research at IIMCB, giving investigators access to a broad spectrum of cutting edge research technology platforms extensively used in such diverse areas as structural biology, bioinformatics, molecular and cell biology. It is being run by experienced scientists who devote part of their time to maintenance of the most sophisticated apparature, being also the top authority in research application of pieces of equipment of their competence. More than 200 items of the equipment are grouped according to applications for biophysical and biochemical methods of protein and nucleic acids structure and function determination (e.g. bioreactor, chromatography stations – 13 of FPLC and 4 of HPLC, centrifuges, ultracentrifuges, analytical ultracentrifuge, crystallization hotels and robots, X-ray generator, spectrophotometers, spectrofluorometers including one with a stopped flow, BIACORE 3000, circular dichroism spectrometer, FT-IR spectrometer, mass spectrometers - MALDI and nanoLC/MS/MS ESI, DNA synthesizer, capillary DNA sequencer, multi angle light scattering detector - MALS, plate readers, RT PCR and others), cell biology techniques (FACS, wide-field and confocal fluorescence microscopes, multi-photon microscope, high throughput system for live cell imaging and intracellular calcium measurements) and isotope methods (imaging systems, scintillation counter). All equipment is staffed and maintained by experienced scientists.

The Laboratory provides sufficient assistance, from method principle, experiment design and initial training through all the procedures needed for an experiment to the data analysis and final interpretation. The use of the equipment is free of charge to all faculty members and students. The Core Facility is also available to scientists from other institutes.

Zebrafish Core Facility

Head: **Małgorzata Wiweger**, PhD

Technician:
Piotr Korzeniowski, DVM, Maciej Mańk, MSc,
Krzysztof Surga, MSc, Monika Turniak, MSc

The Zebrafish Core Facility was opened in November 2012. This licensed breeding and research facility was established as a base for FishMed project (for more information see p. 18) and other biomedical studies. This state-of-the-art facility includes a water plant, as well as a stand-alone quarantine unit and the main system manufactured by Tecniplast. The system can hold approximately 6,000 adult fish and a further expansion is scheduled. At the present time, 25 different lines of zebrafish are being housed and more lines will shortly be introduced. Beside the aquarium, which is a restricted area, our Zebrafish Core Facility has a laboratory space fully equipped for standard fish work and available to all users. Alongside incubators, microscopes and injectors, the laboratory is also equipped with a needle puller and beveller, suited for production of capillary needles for injection of zebrafish, Drosophila and other organisms. The Zebrafish Core Facility is scheduled to be fully operational and ready to supply fish and expertise to both internal and external users in the first half of 2013.

Zebrafish are small tropical fish (3-5cm) with a life-cycle of approximately 3-4 months. External fertilization, translucent body, large mutant/transgenic collection and availability of various genetic tools make zebrafish an excellent organism to study multiple aspects of human diseases. Furthermore, the use of this lower vertebrate animal model opens new possibilities for introducing "3R" (reduction, replacement and refinement) ethical guidance at the Ochota Campus. The use of Zebrafish Core Facility is free of charge to all faculty members and students.

Centre for Innovative Bioscience Education (BioCEN)

The aim of the Centre for Innovative Bioscience Education (BioCEN), is to reduce the gap between science and society in Poland by conducting educational activities popularizing biology: open lectures, workshops for students and courses for biology teachers. All activities are focused on improving biology education and the awareness of biology in society. The co-founders of the Centre for Innovative Bioscience Education are: the International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (IBD), the Institute of Biochemistry and Biophysics PAN (IBB), University of Warsaw's Faculty of Biology, and the BioEducation Foundation. IIMCB houses the BioCEN laboratory, office and administration. BioCEN also coordinates a second laboratory at the Warsaw University of Life Sciences. In 2012 alone, over 2,500 young participants attended laboratory workshops and over 60 biology teachers participated in laboratory workshops and courses. In the span of 11 years of its activity BioCEN was visited by 12,861 students who attended workshops on various topics.

Laboratory workshops

Workshop participants use laboratory equipment and techniques for real-life experiments. Practical experiments are supported by lectures presenting the theoretical basis and techniques of molecular biology and genetics. Each workshop takes four hours over the course of one day. We offered the following topics:

- Explore your own DNA – examining DNA by PCR methods
- Let's play with bacteria – a plasmid isolation and restriction map
- Green bacteria – bacteria transformation with the GFP gene
- Protein fingerprints of different tissues
- Miracles of biotechnology – purification of jellyfish protein from bacteria
- Investigate signs of evolution in your DNA – methods of molecular evolution
- Yeast – a living micro-factory
- Do you know what you eat?
- Biotechnology of antibodies in clinical practice.

In 2012 BioCEN developed two new class topics for students of primary and secondary schools. Both topics concern the knowledge on DNA:

- "On the trail of DNA"
- "See DNA"
- 2012 was also the first year in which senior pupils of primary schools were invited to attend regular lab workshops held during the school year.

Courses for biology teachers

- Since we strongly encourage teachers to implement practical protocols at schools, we equip them with classroom scenarios and affordable experimental kits that can be used in school laboratories. The proposed teaching materials exemplify a state of-the-art approach towards innovative biology education. They allow for the development of practical skills and introduce a teaching approach based on project development by a team of students. Last but not least, our educational procedures improve the ability of analytical thinking. During our workshops we popularize a method known as Inquiry Based Science Education. "Inquiry" is defined as„a search for truth, information, or knowledge" – seeking information by questioning. Although Inquiry Based Science Education can be applied to all disciplines, it is especially important in science education.

In 2012, as part of teacher training, the following events were organized:

- 11th BioCEN and Nencki Institute Symposium for teachers.
- Training for 28 teachers in the project, "I understand, because I experiment – experimental kits for junior high schools"

16th Science Picnic (May 12, 2012)

As in previous years, the BioEducation Foundation and BioCEN organized an exhibition and science show during the 16th Science Picnic in Warsaw. The motto for 2012 was "Energy".

One of our demonstrations was related to DNA and the variety of methods used in molecular biology research:

- Necklaces with your own DNA – isolation of DNA from a cheek swab

The second experiment illustrated yeast's ability to produce electricity.

16th Science Festival (September 22-29, 2012)

The objective of the Warsaw Science Festival is to enhance public awareness of science and technology. Over 500 activities take place in different formats (seminars, debates, guided tours, workshops, performances, contests, films), representing various fields of science.

They are aimed at different target groups (young children, primary school, high school, general public) and are run for one week at various venues: universities, scientific research institutions and museums. In 2012 BioCEN organized open laboratory workshops for the public:

- "See DNA?" a workshop for students
- "On the trail of DNA" a workshop for students

10th anniversary of Centre for Innovative Bioscience Education (September 22, 2012)

As part of BioCEN's 10th anniversary we organized an open-day event entitled "Open Laboratory of Molecular Biology" and invited anyone who wanted to visit us. Throughout the day our Open Laboratory was visited by over 200 people to whom we presented the techniques of molecular biology, achievements in biotechnology, genetically modified organisms and the analysis of DNA with the use of agarose gels.

6th Children's Science Festival (September 29, 2012)

It was the second time when BioCEN participated in the Children's Science Festival. During several hours of workshops children learned about the yeast respiration process and investigated the properties of the juice of red cabbage. Several hundred children took part in the workshop.

Family laboratory workshops

In 2012 we continued to develop laboratory workshops for younger children, accompanied by their guardians. Many years of working with children has enabled us to develop a unique program of educational workshops tailored to their age. During our workshop the little scientists perform each experiment themselves, under the supervision of an experienced tutor. Guardians accompany the children and take part in carrying out experiments. Currently, we offer four different workshop topics to families who visit our laboratory, including:

- How many vitamins are there in a candy?
- Why don't plants need to eat?

Evaluation of the workshop by the Institute for Educational Research (IBE)

In 2012, an evaluation of our educational offer was carried out by the Institute for Educational Research. We were chosen for the analysis out of 348 educational centres across Poland. The evaluation indicated that our activities met the majority of Best Practices in Education requirements, defined by the IBE. These include:

- providing significant support for schools in implementing the requirements of the new core curriculum, e.g. explaining the rules of "scientific thinking",
- implementing experiments and observations,
- learning to draw conclusions,
- determining cause-and-effect relationships,
- working in small groups,
- offering classes conducted by young and enthusiastic animators.

In the post-classes surveys, teachers claimed that they definitely would like to attend classes again. Students, on their part, indicated in their survey that during workshops they had felt like real researchers performing laboratory experiments and they found workshops interesting.

Media in the laboratory

Twice in 2012 BioCEN hosted a Polish TV program "How It Works". During the first show the experiments on yeast were performed and the second show focused on basic experiments on DNA.

The Center for Innovative Bioscience Education – partner of the Center for Citizenship Education in the Project "The Students Academy" co-founded by the European Coherence Fund (EFS)

"The Students' Academy" is an initiative which brings together 300 junior high schools and 35 thousand students from five regions of Poland. During workshops students design and carry out experiments and team projects and make observations in accordance with scientific procedures. Teachers from participating schools have access to Internet-based coaching and participate in professional training, focusing on the preparation of scientific observations and experiments for students, guidance for student projects, and approaches to motivate learning. The project started in 2010 and will continue for 4 years. BioCEN monitors and verifies the accuracy of biology teachers' ideas and experimental scenarios and oversees the accuracy of the biological experiments conducted by students.

Staff and co-workers

Persons who coordinate and administrate BioCEN are: Agnieszka Chołuj, Karolina Kurzela, Aleksandra Kot-Horodyńska, Marcin Wiśniewski (as a coordinator at Warsaw University of Life Sciences) Animators and co-workers: Kamil Koper, Marek Kulka, Marta Strumiłło, Piotr Horodyński, Ewa Podobas, Zuzanna Sobańska, Michał Mlącki, Maciej Lirski, Aleksandra Piechnik, Paulina Mrozek, Marek Krzyżanowski, Michał Spanier, Jan Zawitkowski, Katarzyna Laskowska – Kaszub, Aleksandra Kwiatkowska, Dominika Strzelecka, Kryspin Andrzejewski, Anna Sapeta, Zuzanna Maniecka, Ada Rejch, Karolina Malanowska, Justyna Rudzka, Jacek Patryn, Katarzyna Krzyczmonik, Gabriela Matuszko, Katarzyna Łepeta

Educational Activities

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus. Currently 50 PhD students are on board within the doctoral programs of the Institute of Biochemistry and Biophysics, of the Nencki Institute, of the University of Poznań and of the Foundation for Polish Science.

The PhD students of IIMCB are self-organized as a group with the representative Marcin Magnus. They have regular working seminars every two months. The postdoctoral fellows are similarly self-organized with group representatives Elżbieta Purta and Karolina Górecka. The "Postdoc's seminars" are devoted to the presentation of personal experience of lecturer, being complementary to regular IIMCB seminars. Both groups representatives participate in meetings with Directors, Lab Leader's meetings, etc.

International PhD Programme

This program started in 2010 based on funds of the Foundation for Polish Science. PhD projects are being realized in the Institute of Biochemistry and Biophysics PAN and in the International Institute of Molecular and Cell Biology, in collaboration with a number of foreign partner institutions. PhD projects were made available in the major areas of molecular biology, like DNA metabolism, RNA biogenesis and its control, mechanisms of cellular signalling and trafficking and in applied molecular biology field; seven of them were affiliated with IIMCB:

1. *Identification and characterization of novel nucleases*
   Supervisor: Janusz Bujnicki
   Foreign partner: Ichizo Kobayashi (Japan)
2. *mTor regulated cellular trafficking in neuronal development*
   Supervisor: Jacek Jaworski
   Foreign partner: Casper Hoogenraad (The Netherlands)
3. *High throughput detection of calcium homeostasis for AD diagnosis and drug discovery based on interaction between STIM protein and plasma membrane calcium channels*
   Supervisor: Jacek Kuźnicki
   Foreign partner: Jochen Herms (Germany)
4. *Endocytic trafficking and intracellular signaling of PDGF ligands and receptors*
   Supervisor: Marta Miączyńska
   Foreign partner: Carl-Henrik Heldin (Sweden)
5. *Structural studies of DNA substrate binding by the GYI-IYG domain*
   Supervisor: Marcin Nowotny
   Foreign partner: Titia K. Sixma (The Netherlands)
6. *Studies of genetic basis of ciliopathies*
   Supervisor: Michał Witt
   Foreign partner: Heymut Omran (Germany)
7. *Molecular mechanism of oncogenic activity of p53 gain of function cancer mutants*
   Supervisor: Alicja Żylicz
   Foreign partner: Ted Hupp (UK)

Support for bio tech med scientists in technology transfer

In 2012 IIMCB was running several projects to support technology transfer in Biocentrum Ochota consortium.

Projects:

- "Effective Technology Transfer in Biotechnology, ETTBio", supported with the funds from the INTERREG IVC programme;
- "Support for bio-tech-med scientists in technology transfer through scholarships, training courses and internships", sponsored by Operational Programme - Human Capital; 8.2.1
- "Support for the protection of industrial property generated in scientific entities as a result of R&D work", supported by Operational Programme – Innovative Economy 1.3.2.
- Kreator Innowacyjności, the programme of the National Centre for Research and Development

Several activities were made possible within these projects:

- research stipends for innovative projects for PhD students working in Biocentrum Ochota institutes,
- two-month practices for Biocentrum Ochota scientists at industrial sites,
- training courses on issues such as: R&D project management, raising a company, commercialization of R&D results, Intelectual Property Rights (IPR), negotiations in business,
- joint science to business projects
- funds secured for patent protection
- management of IP and commercialization of R&D results

Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS) at the University of Gdańsk

IIMCB has joined LiSMIDoS program with a major goal to participate in activities of independent Doctoral School, with real influence on its educational curriculum: in this framework IIMCB faculty will run courses, summer schools, etc. Some of them will be performed in a teleconference format. The major objective is to provide a programme of interdisciplinary training to PhD students that will allow them to work in today's competitive scientific environment that very often requires crossdisciplinary expertise.

Administration

Scientific Office

Dominika Dubicka-Boroch Director's Assistant
Agnieszka Wagner-Ziemka Domestic Cooperation Manager
Katarzyna Dąbrowska Domestic Grants Administrator

Administration Unit

Agnieszka Karbowska Director's Representative for Administrative Matters
Roman Szczepanowski Director's Representative for Information Technology and Research Equipment
Anna Brzezińska Tenders Specialist
Agnieszka Gwara Secretary (since March 2013)
Dorota Makulska Secretary (until Feb. 2013)
Robert Banasiak Maintenance Specialist

International Cooperation Unit

Aleksandra Nałęcz-Tolak International Cooperation Specialist
Urszula Białek-Wyrzykowska International Cooperation Manager
Dorota Libiszowska Foreign Grants Specialist
Marcin Ogonowski International Cooperation Specialist

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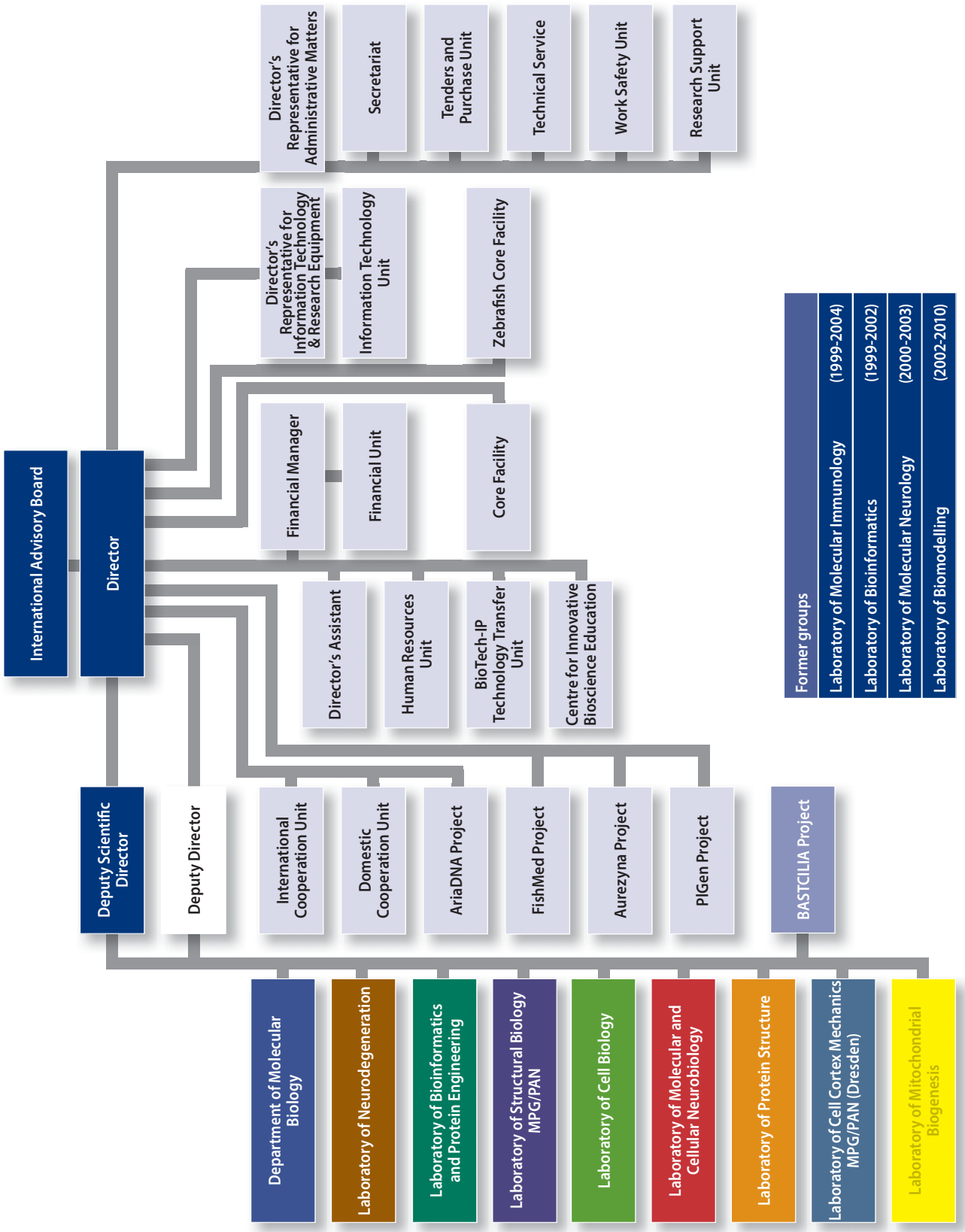
Hanna Iwaniukowicz Financial Manager
Renata Knyziak Accounting Specialist
Agnieszka Kuna Accounting Specialist
Mariola Arkuszewska Accounting Specialist
Monika Nowicka Payroll Specialist

Human Resources Unit

Monika Domańska-Paśko Human Resources Specialist
Beata Tkacz Human Resources Manager

Structure of the International Institute of Molecular and Cell Biology



Staff at IIMCB (as of 31 March 2013)

Administration		Funding
Jacek Kuźnicki	Director	IIMCB
Michał Witt	Deputy Scientific Director	IIMCB (1/2)
Hanna Iwaniukowicz	Financial Manager	IIMCB
Monika Nowicka	Payroll Specialist	IIMCB
Renata Knyziak	Accounting Specialist	IIMCB
Agnieszka Kuna	Accounting Specialist	IIMCB/Structural Funds
Mariola Arkuszewska	Accounting Specialist	IIMCB/Structural Funds
Beata Tkacz	Human Resources Specialist	IIMCB
Monika Domańska-Paśko	Human Resources Specialist	IIMCB (1/2)
Urszula Białek-Wyrzykowska	International Cooperation Manager	IIMCB (1/2)
Dorota Wasiak-Libiszowska	Foreign Grants Specialist	IIMCB/EC grant
Marcin Ogonowski	International Cooperation Specialist	IIMCB/Structural Funds/EC grant
Aleksandra Nałęcz-Tolak	International Cooperation Specialist	IIMCB/EC grant
Agnieszka Wagner-Ziemka	Domestic Cooperation Manager	IIMCB
Katarzyna Nakielska	Domestic Grants Administrator	NCBiR grant
Agnieszka Karbowska	Director's Representative for Administrative Matters	IIMCB
Roman Szczepanowski	Director's Representative for Information Technology and Research Equipment	IIMCB (2/3)
Dominika Dubicka	Director's Assistant	IIMCB
Anna Brzezińska	Tender Specialist	IIMCB
Agnieszka Gwara	Secretary	IIMCB
Robert Banasiak	Maintenance Specialist	IIMCB
Dudzin Magdalena	Work Safety Specialist	IIMCB (1/4)

Core Facility		
Alicja Żylicz	Head	IIMCB
Roman Szczepanowski	Senior Staff Scientist	IIMCB (1/3)
Krzysztof Skowronek	Senior Staff Scientist	IIMCB (1/2)
Tomasz Węgierski	Senior Staff Scientist	IIMCB (1/2)
Piotr Brągoszewski	Radiation Safety Specialist	IIMCB (1/2)

Zebrafish Core Facility		
Małgorzata Wiweger	Head	EC grant
Piotr Korzeniowski	technician	EC grant (1/2)
Monika Turniak	technician	EC grant (1/2)
Maciej Mańk	technician	EC grant (1/2)
Krzysztof Surga	technician	EC grant (1/2)

Department of Molecular Biology		
Maciej Żylicz	Head	IIMCB (1/2)
Bartosz Wawrzynów	Senior Researcher	IIMCB
Marta Małuszek	Junior Researcher	IBB PhD School
Magdalena Pruszko	Junior Researcher	FNP International PhD programme
Zuzanna Tracz-Gaszewska	Junior Researcher	IBB PhD School/FNP Ventures Programme
Milena Wiech	Junior Researcher	Nencki PhD School/NCN Preludium Programme
Grażyna Orleańska	Secretary	IIMCB (1/2)

Laboratory of Cell Biology		
Marta Miączyńska	Head	IIMCB
Magdalena Banach-Orłowska	Postdoctoral Fellow	Structural Funds/FNP POMOST
Anna Bartosik	Postdoctoral Fellow	EC grant
Beata Pyrzyńska	Postdoctoral Fellow	Polish-Swiss Fund
Ewelina Szymańska	Postdoctoral Fellow	Structural Funds/FNP POMOST
Lidia Wolińska	FishMed Technical Assistant	EC grant (1/2)
Kamil Jastrzębski	Junior Researcher	FNP
Agnieszka Mamińska	Junior Researcher	IIMCB
Sam D. Stephen	Junior Researcher	EC grant
Anna Toruń	Junior Researcher	IIMCB
Izabela Zacharek	Grant Administrator and Lab Manager	Polish-Swiss Fund/ Team FNP

Laboratory of Bioinformatics and Protein Engineering		
Janusz M. Bujnicki	Head	IIMCB/ERC grant
Michał Boniecki	Postdoctoral Fellow	DFG (International funds)
Grzegorz Chojnowski	Postdoctoral Fellow	IIMCB/ TEAM FNP
Stanisław Dunin-Horkawicz	Postdoctoral Fellow	EXGENOMES
Bogusław Kluge	Postdoctoral Fellow	ERC grant/NCN grant
Grzegorz Łach	Postdoctoral Fellow	IIMCB/ TEAM FNP
Martyna Nowacka	Postdoctoral Fellow	NCN grant
Elżbieta Purta	Postdoctoral Fellow	IIMCB/ TEAM FNP
Krzysztof Skowronek	Postdoctoral Fellow	ERC grant
Tomasz Waleń	Postdoctoral Fellow	ERC grant
Piotr Bentkowski	Postdoctoral Fellow	IIMCB
Krzysztof Szczepaniak	Junior Researcher	NCN grant
Sylwia Panek	Technican	ERC grant
Justyna Czarnecka	Technican	ERC-PoC grant
Astha	Junior Researcher	NCN grant
Magdalena Byszewska	Junior Researcher	NCN grant
Ilona Domagała	Junior Researcher	Structural Funds (Bio&Techn. Innov.)
Dawid Głow	Junior Researcher	NCN grant
Katarzyna H. Kamińska	Junior Researcher	(part time 25% in LBPE, until December 2013 on intership in IGHT s.c. Company) IIMCB
Łukasz Kozłowski	Junior Researcher	EXGENOMES
Małgorzata Kurkowska (Durawa)	Junior Researcher	NCN grant
Magdalena Machnicka (Mika)	Junior Researcher	TEAM FNP
Marcin Magnus	Junior Researcher	TEAM FNP
Dorota Matelska	Junior Researcher	TEAM FNP
Shamba Sankar Mondal	Junior Researcher	TEAM FNP
Anna Olchowik	Junior Researcher	Structural Funds(MPD Project)
Jakub Jopek	Junior Researcher (co-supervision)	Structural Funds (funded by a PhD school of Warsaw Univ.)
Paweł Piątkowski	Junior Researcher	TEAM FNP
Agata Sulej (Kamaszewska)	Junior Researcher	ERC-PoC grant
Juliusz Stasiewicz	Junior Researcher	TEAM FNP
Irina Tuszyńska	Junior Researcher	ERC grant/NCN grant
Maria Werner	Junior Researcher	NCN grant
Albert Bogdanowicz	MSc Student	TEAM FNP
Witold Januszewski	MSc Student	TEAM FNP
Rafał Zaborowski	MSc Student	TEAM FNP
Agnieszka Faliszewska	Office Manager	IIMCB/ TEAM FNP
Jan Kogut	Computer Administrator/Programmer	Structural Funds (BIOCENTRUM) (1/2)
Tomasz Jarzynka	Computer Administrator/Programmer	Structural Funds (BIOCENTRUM) (1/2)
Łukasz Munio	Computer Administrator	Structural Funds (BIOCENTRUM)

Laboratory of Neurodegeneration		
Jacek Kuźnicki	Head	IIMCB
Urszula Wojda	Associate Professor, Vice Head	IIMCB
Tomasz Węgierski	Senior Researcher	IIMCB (1/2)
Marta Wiśniewska	Senior Researcher	Era-Net Neuron
Magdalena Czeredys	Postdoctoral Fellow	EC grant
Joanna Gruszczyńska-Biegała	Postdoctoral Fellow	IIMCB/NCN grant
Łukasz Majewski	Postdoctoral Fellow	NCN grant
Smijin Karthully Soman	Postdoctoral Fellow	EC grant (since March 2013)
Kinga Gazda	Junior Researcher	NCN grant
Anna Jaworska	Junior Researcher	FNP
Kamil Koper	Junior Researcher	Ministerial Grant
Łukasz Szewczyk	Junior Researcher	Era-Net Neuron
Andrzej Nagalski	Junior Researcher	IIMCB
Katarzyna Sawicka	Junior Researcher	NCN grant
Aleksandra Szybińska	Junior Researcher	IIMCB
Nikola Brożko	Junior Researcher	Ministerial Grant
Michal Bazała	FishMed Technical Assistant	EC grant (1/2)
Anna Piotrowska	Secretary	IIMCB (1/2)

Laboratory of Structural Biology		
Matthias Bochtler	Head	IIMCB
Honorata Czapińska	Postdoctoral Fellow	TEAM FNP (3/4)
Monika Sokołowska	Postdoctoral Fellow	Ministerial grant
Marek Wojciechowski	Postdoctoral Fellow	IIMCB
Katrzyna Misztal	Postdoctoral Fellow	IIMCB
Agnieszka Kolano	Postdoctoral Fellow	EC grant
Patrycja Haniewicz	Junior Researcher	Ministerial grant
Asgar Abbas Kazrani	Junior Researcher	FNP
Karolina Mierzejewska	Junior Researcher	FNP
Dominik Rafalski	Junior Researcher	FNP
Karthik Shanmuganandam	Junior Researcher	FNP
Wojciech Siwek	Junior Researcher	NCN grant
Mateusz Kostecki	MSc Student	Volunteer

Laboratory of Molecular and Cell Neurobiology		
Jacek Jaworski	Head	IIMCB
Magdalena Błażejczyk	Postdoctoral Fellow	Era-Net Neuron (1/2)
Iwona Cymerman	Postdoctoral Fellow	NCN grant
Agata Góźdź	Postdoctoral Fellow	NCN grant/Era-Net Neuron grant
Matylda Macias	Postdoctoral Fellow	NCN grant
Ewa Liszewska	Postdoctoral Fellow	Era-Net Neuron
Bartosz Tarkowski	Postdoctoral Fellow	NCN grant
Anna Malik	Postdoctoral Fellow	NCN grant
Joanna Lipka	Junior Researcher	FNP
Agnieszka Skałecka	Junior Researcher	NCBiR
Anna Urbańska	Junior Researcher	IIMCB
Malgorzata Urbańska	Junior Researcher	IIMCB
Adrianna Łach	MSc Student	Volunteer
Anna Bajur	MSc Student	Volunteer
Marcelina Pieprzyk	Technician	Era-Net Neuron/NCBiR
Lidia Wolińska	FishMed Technical Assistant	EC grant (1/2)

Laboratory of Protein Structure		
Marcin Nowotny	Head	Wellcome Trust/UE
Elżbieta Nowak	Postdoctoral Fellow	EC grant
Vinnet Gaur	Postdoctoral Fellow	Wellcome Trust
Karolina Górecka	Postdoctoral Fellow	Wellcome Trust
Jakub Gruchota	Postdoctoral Fellow	HHMI
Aleksandra Knapik	Assistant	HHMI
Marcin Jaciuk	Junior Researcher	ERC
Mirosław Śmietański	Junior Researcher	HHMI
Michał Miętus	Junior Researcher	FNP
Magdalena Łazęcka	Lab Manager	Wellcome Trust
Monika Rychlik	Lab Manager	Wellcome Trust
Marzena Nowacka	Technician	EC grant
Weronika Komorowska	Technician	Ministerial Grant
Paweł Kustosz	Technician	EC grant
Agnieszka Olszewska	Technician	IIMCB
Iwona Ptasiewicz	Technician	IIMCB
Justyna Studnicka	Technician	Wellcome Trust

Laboratory of Mitochondrial Biogenesis		
Agnieszka Chacińska	Head	IIMCB
Piotr Brągoszewski	Postdoctoral Fellow	Welcome FNP/IIMCB
Anna Sokół	Postdoctoral Fellow	EC
Małgorzata Sztolsztener	Postdoctoral Fellow	Welcome FNP/EMBO IG
Michał Wasilewski	Postdoctoral Fellow	NCN/IIMCB
Ulrike Topf	Postdoctoral Fellow	Swiss NSF
Edyta Głów	PhD Student	Welcome FNP
Agnieszka Górnicka	PhD Student	Welcome FNP
Paulina Kwiatkowska	PhD Student	EMBO IG
Aksana Varabyova	PhD Student	NCN
Lidia Wróbel	PhD Student	Welcome FNP
Anita Brewińska	Research Assistant	Welcome (1/2)
Magdalena Chojnacka	MSc Student	Welcome FNP
Michał Bazała	FishMed Technical Assistant	EC (1/2)

Aurezyna Project		
Izabela Sabała	Coordinator	NCBiR grant
Elżbieta Jagielska	Postdoctoral Fellow	NCBiR grant
Maja Grabowska	Junior Resercher	NCBiR grant
		NCBiR grant

PlGen Project		
Małgorzata Mossakowska	Coordinator	NCBiR grant
Aleksandra Szybalska	Project Assistant	NCBiR grant
Przemysław Ślusarczyk	IT Specjalist	NCBiR grant
Katarzyna Wodzyńska	Assistant	NCBiR grant

Research Support Unit		
Wanda Gocal	Technician	IIMCB
Elżbieta Grzelak	Technician	IIMCB
Monika Matuszczyk	Technician	IIMCB
Iwona Ptasiewicz	Technician	IIMCB
Alina Zielińska	Technician	IIMCB

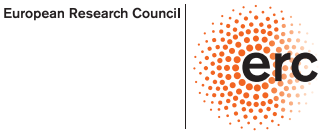
Technology Transfer Unit (Biotech-IP)		
Magdalena Powierża	Head	Structural Funds
Adam Sobczak	Project Manager	Structural Funds (1/2)
Leszek Lipiński	Industrial Cooperation Manager	Structural Funds (1/2)
Agnieszka Banrowska	Technology Transfer Manager	NCBiR grant
Piotr Potepa	Technology Transfer Assistant	Structural Funds
Hubert Ludwiczak	Technology Transfer Assistant	Structural Funds (1/4)

Centre for Innovative Bioscience Education		
Agnieszka Chołuj	Head	CEO (projekt)
Karolina Kurzela	Coordinator	IBB/Dept. of Biology/IIMCB
Aleksandra Kot-Horodyńska	Coordinator	Nencki
Marcin Wiśniewski	Coordinator	SGGW
Kamil Koper		Volunteer
Michał Mlącki		Volunteer
Marek Kulka		Volunteer
Marta Strumiłło		Volunteer
Piotr Horodyński		Volunteer
Ewa Podobas		Volunteer
Zuzanna Sobańska		Volunteer
Maciej Lirski		Volunteer
Aleksandra Piechnik		Volunteer
Paulina Mrozek		Volunteer
Marek Krzyżanowski		Volunteer
Michał Spanier		Volunteer
Jan Zawitkowski		Volunteer
Katarzyna Laskowska-Kaszub		Volunteer
Aleksandra Kwiatkowska		Volunteer
Maciej Szajnach		Volunteer
Dominika Strzelecka		Volunteer
Kryspin Andrzejewski		Volunteer
Anna Sapeta		Volunteer
Zuzanna Maniecka		Volunteer
Ada Rejch		Volunteer
Karolins Malanowska		Volunteer
Justyna Rudzka		Volunteer
Jacek Patryn		Volunteer
Katarzyna Krzyczmonik		Volunteer
Gabriela Matuszko		Volunteer
Katarzyna Łepeta		Volunteer

Important Dates in the Institute's History

Sept. 1991	The proposal to create the Institute was published in the UNESCO Bulletin of MCBN
June 1994	State Committee for Scientific Research (KBN) accepts the activities aimed at establishing the Institute
Oct. 1994	Presidium of Polish Academy of Sciences (PAN) votes to support the Institute
May 1995	An agreement between Poland and UNESCO to establish the Institute
June 1996	The Molecular and Cell Biology Department is created by PAN with Prof. M.J. Nałęcz as the Head
June 1997	Polish Parliament passes a bill to found the Institute
May 1998	Prof. A. Azzi is nominated as the Director of IIMCB
Jan. 1999	The Institute commences its independent activities; Prof. J. Kuźnicki appointed as Acting Director
July 1999	Dr. J. Dastych is appointed as a first Lab Leader at IIMCB
Oct. 1999	Prof. M. Żylicz is appointed as Chair of the Department of Molecular Biology
April 2000	An agreement between the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN) to launch a Joint MPG-PAN Junior Research Group
Jan. 2001	The MPG-PAN Junior Research Group commences its activities with Dr. M. Bochtler as a Lab Leader
June 2001	Prof. J. Kuźnicki is elected by the International Advisory Board as Director of the Institute, begins to complete the Laboratory of Neurodegeneration. After consultation with UNESCO, the official nomination was signed by the President of PAN on February 1, 2002
Nov. 2002	New members of the International Advisory Board nominated for 2002-2006 term
Jan. 2003	Status of the Centre of Excellence in Molecular Bio-Medicine is granted by the European Commission within 5th Framework Programme
June 2005	Professor J. Kuźnicki re-elected as Director of the Institute (term 2006-2010)
May 2006	New members of the International Advisory Board nominated for 2006-2010 term
Feb. 2006	Twin MPG-PAN laboratory established at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden with Dr. Ewa Paluch as a Lab Leader
May 2009	Professor J. Kuźnicki re-elected as Director of the Institute (term 2010-2014)
Jan. 2010	New members of the International Advisory Board nominated for 2010-2014 term

Funding Institutions



FISHING FOR MEDICINES AND THEIR TARGETS

USING ZEBRAFISH MODELS OF HUMAN DISEASES

FISH MED

Project by the International Institute of Molecular and Cell Biology

International Institute of Molecular and Cell Biology in Warsaw invites to a kick-off meeting of the FishMed project.
The meeting will be held on April 12th 2013, 4 Ks. Trojdena Street.

GENERAL PART (lecture hall IBIB)

09.00-09.10 Representative of the Ministry of Science and Higher Education
09.10-09.30 Grzegorz Ambroziewicz, European Commission - Horizon2020
09.30-09.40 Jacek Kuźnicki - presentation of the FishMed project
09.40-10.00 Ewa Snaar-Jagalska, Leiden University, The Netherlands - an introductory lecture
10.00-10.30 Press briefing / Coffee break

SCIENTIFIC SYMPOSIUM (lecture hall IBIB)

10.30-11.10 **Molecular circuits controlling myocardial remodeling and regeneration**
Thomas Braun, Max-Planck-Institute for Heart and Lung Research, Germany

11.10-11.50 **Using zebrafish to study retinal development**
William Harris, Cambridge University, United Kingdom

11.50-12.30 **Drug screens in an academic environment – how far have we got?**
Oliver Bandmann, University of Sheffield, United Kingdom

12.30-13.10 **Zebrafish: a new engraft model for in vivo studies on human cancers**
Ewa Snaar-Jagalska/Herman P. Spaink, Leiden University, the Netherlands

13.10-14.10 Lunch

14.10-14.50 **Imaging heart development and function in zebrafish**
Didier Stainier, Max-Planck-Institute for Heart and Lung Research, Germany

14.50-15.30 **Cell and tissue mechanics in zebrafish gastrulation**
Carl-Philipp Heisenberg, Austrian Institute of Science and Technology, Austria

15.30-16.10 **Sara endosomes and asymmetric cell division**
Marcos Gonzalez-Gaitan, University of Geneva, Switzerland

PROJECT MEETING (closed session)

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

SEVENTH FRAMEWORK PROGRAMME

Project supported by the EC FP7 grant FishMed, GA No 316125

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