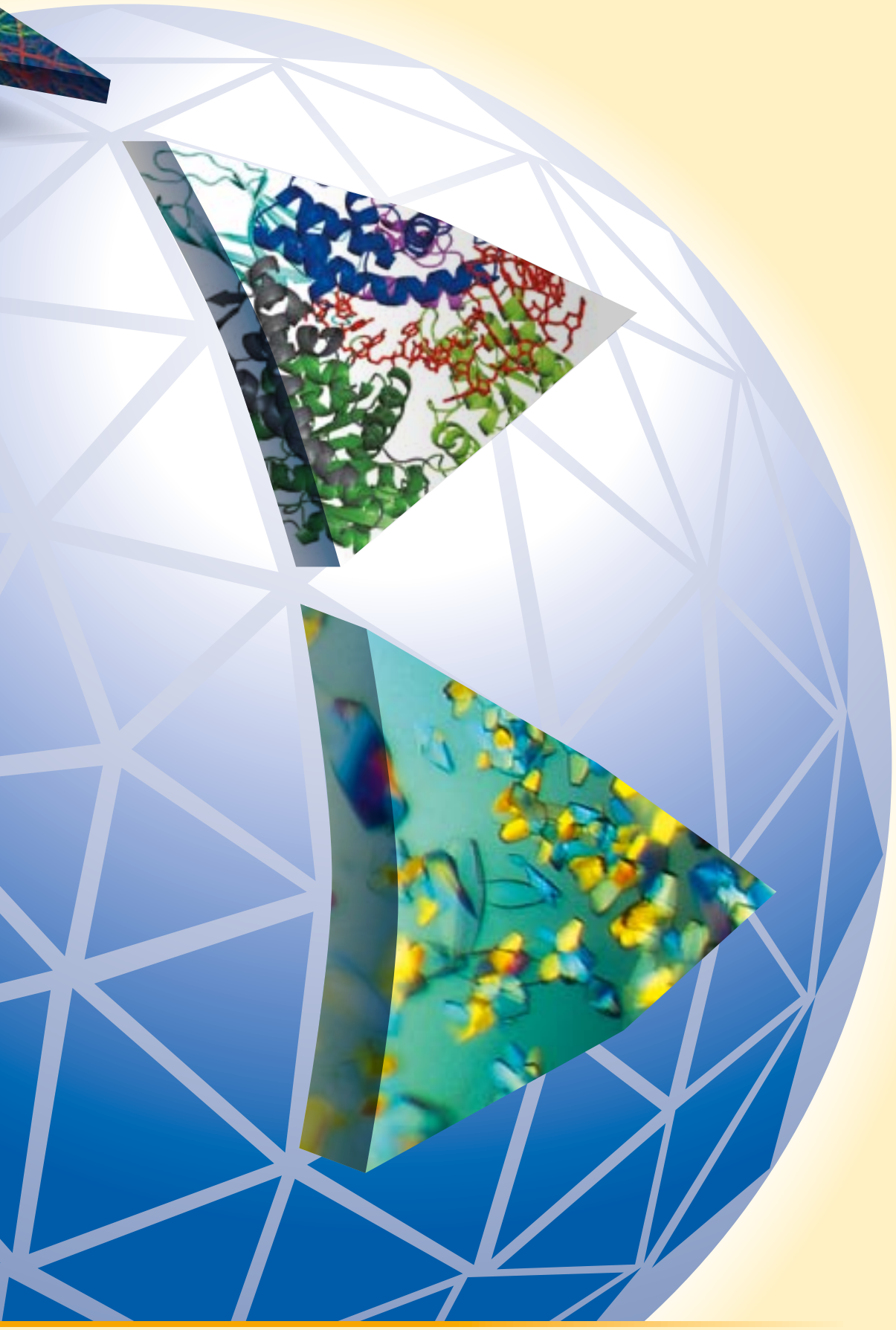
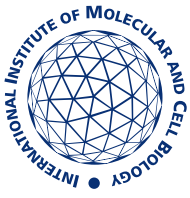


Annual Report 2009

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY



Director
Jacek Kuźnicki

Deputy Scientific Director
Michał Witt

Financial Manager
Hanna Iwaniukowicz

Chairman of the International Advisory Board
Angelo Azzi

Deputy Chairman of the International Advisory Board
Leszek Kaczmarek

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Agnieszka Wagner-Ziemka and Michał Witt

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Contents

10th Anniversary of IIMCB

From left to right: Ganna Jelskaja, Jacek Kuźnicki i Natalia Shulga during the Polish-Ukrainian poster session; Malgorzata Mossakowska receiving honorary diploma as the first employee of IIMCB (above); anniversary cake (below); anniversary balloons held by our administration staff.

The 10th anniversary of the IIMCB was not merely a festive event. In May 2009 we celebrated the end of the first decade of the IIMCB in a fashion which was as scientific as it was entertaining, with the anniversary dinner being very much just an addition to the proceedings. On this occasion the IIMCB organized an international research symposium – a kick-off meeting of the HEALTH-PROT project presenting the project partners and potential partners of other European projects, with lectures delivered by: Wolfgang Zachariae, Vincenza Andrisano, Saulius Klimasauskas, Ted Hupp, Anita Becker, Samantha Spangler and Oleg Krishtal. It was preceded by a Polish-Ukrainian research collaboration meeting, where the IIMCB hosted a group of 23 Ukrainian scientists. The event was held in a form of a poster session, with 18 posters from the Institute of Molecular Biology and Genetics in Kiev and 18 posters from the IIMCB. The Ukrainian group was led by Prof. Ganna Jelskaja, Director of the Institute of Molecular Biology and Genetics in Kiev and Dr. Natalia Shulga, CEO at the Ukrainian Scientific Club. The anniversary party followed, complete with a cake shaped like the Institute's logo.

It all dates back to 1994, when the newly established Committee for Scientific Research (KBN) and the Presidium of the Polish Academy of Sciences approved a UNESCO initiative resulting in an international agreement signed in May 1995 by Prof. F. Mayor, UNESCO's Director General, and Prof. A. Łuczak, Poland's Deputy Prime Minister and Head of KBN. The agreement established the International Institute of Molecular and Cell Biology (IIMCB) in Warsaw. This formally fulfilled previous plans of: Prof. Angelo Azzi, Prof. Maciej Nałęcz, Prof. Jacek Kuźnicki and Prof. Leszek Kaczmarek, later to be joined by Prof. Ryszard Przewłocki and Prof. Michał Witt. At the same time, the Polish Parliament (Sejm) and Poland's President ratified the May 1995 agreement. The correct legal foundation for the operations of the IIMCB was finally created by the Parliamentary bill of 26 June 1997 – until then the Polish legal system had been unable to accommodate scientific institutions of international standing. Prof. Angelo Azzi was formally appointed as Head of the Institute, with deputies Prof. Jacek Kuźnicki (as Acting Director) and Prof. Michał Witt, and the first employee was Dr. Małgorzata Mossakowska.

Finally, on 1 January 1999, the International Institute for Molecular and Cell Biology began its independent existence. In the same year, the first professorial positions were filled and two research laboratories were launched: the Laboratory of Molecular Immunology, headed by Dr. Jarosław Dastych, and the Department of Molecular Biology, under the leadership of Prof. Maciej Żylicz. Since 2002, Prof. J. Kuźnicki has been the Director of the Institute, and Prof. M. Witt the Deputy Scientific Director.

The principles of organization of the Institute differ from other research institutes in the country: an important body of the Institute is the International Advisory Board (IAB) which acts as the Scientific Council; the leader of each research team of the Institute is selected in an international competition; each group leader's initial employment is limited to a five-year contract; and three years after the start of research work the progress of research is assessed by the IAB. Based on the IAB's recommendation, a professor's contract may be either terminated or extended.

The IIMCB 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 the members of the IAB and the Institute's Directors. An important link between the Institute and the President of PAN is the 2nd Department of Biological Sciences of PAN, to which the Institute belongs along with sixteen PAN institutes.

The IIMCB 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, Foundation for Polish Science, UNESCO, Framework Programs of EU, Max Planck Society, Howard Hughes Medical Institute, European Molecular Biology Organisation, National Institutes of Health, Wellcome Trust, etc.). About 70% of funds arrive as competitive grant awards received by the group leaders. The IIMCB is located in a building loaned by the Polish Academy of Sciences.

The prospects for the second decade of the International Institute of Molecular and Cell Biology look promising.

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. Nalecz 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.

Directors and Administration

Jacek Kuźnicki
Director

Agnieszka Ziemka
Director's Representative for Research Management

Agnieszka Karbowska
Director's Representative for Administrative Matters

Roman Szczepanowski
Director's Representative for Information Technology & Research Equipment

Zbigniew Przygoda
Director's Advisor (until Dec.2009)

Dominika Dubicka-Boroch
Director's Assistant

Aleksandra Olejniczak
Secretary (until March 2010)

Michał Witt
Deputy Scientific Director

Beata Tkacz
Human Resources Specialist

Krystyna Domańska
Human Resources Specialist

Anna Brzezińska
Tenders Specialist

Urszula Białek--Wyrzykowska
International Cooperation Manager

Dorota Libiszowska
Foreign Grants Specialist

Magdalena Powierża
International Cooperation Specialist

Marcin Ogonowski
International Cooperation Specialist

Hanna Iwaniukowicz
Financial Manager

Monika Nowicka
Payroll Specialist

Renata Knyziak
Accounting Specialist

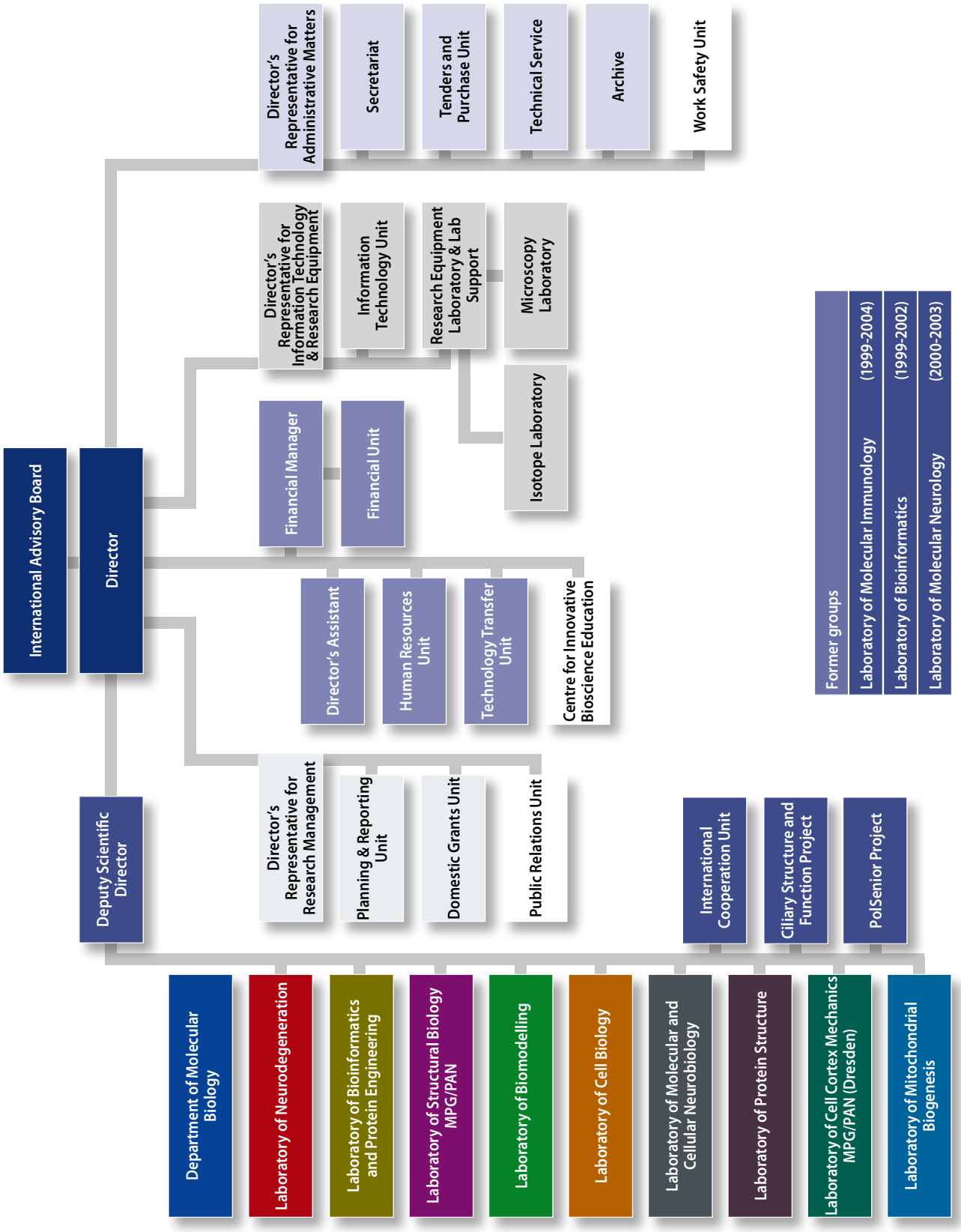
Mariola Arkuszewska
Accounting Specialist

Marcin Biedacha
IT Manager (until Jan. 2010)

Jakub Skaruz
IT Specialist

Robert Banasiak
Maintenance Specialist

Structure of the International Institute of Molecular and Cell Biology



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

2006-2010 term

Participants of the meeting of the International Advisory Board, May 2009
From left (first row): K. Hahlbrock, A. Tramontano, J. Kuźnicki, A. Azzi; (second row) M. Witt, R. Przewłocki, I. Braakman, J.G. Sutcliffe; (third row) I. Dikič, R.P. Erickson, N. Blin, W. Huttner, M.J. Nałęcz, O.A. Krishtal.

Chairman: Angelo Azzi

Deputy Chairman: Leszek Kaczmarek

Members:

Angelo Azzi. Professor, Vascular Biology Laboratory, Tufts University, Boston, MA, USA

Francisco E. Baralle. Director-General of International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Alexey A. Bogdanov. Head of Department of Chemistry and Biochemistry of Nucleoproteins, Department of Chemistry, Moscow State University, Moscow, Russia

Nicolaus Blin. Professor of Molecular Genetics, Institute of Human Genetics, University of Tübingen, Tübingen, Germany; Foreign member of Polish Academy of Sciences

Ineke Braakman. Professor, Department of Cellular Protein Chemistry, Utrecht University, Utrecht, The Netherlands

Ivan Dikič. Professor of Biochemistry, Institute of Biochemistry II, Goethe University Medical School, Frankfurt am Main, Germany

Jerzy Duszyński. Undersecretary of State, Ministry of Science and Higher Education, formerly Director, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Robert P. Erickson. Professor, Department of Pediatrics, Section of Medical and Molecular Genetics, University of Arizona Health Sciences Center, Tucson, AZ, USA

Klaus Hahlbrock. Professor Emeritus, Max-Planck Institute for Plant Breeding Research, Köln, Germany; Laureate of Alexander von Humboldt Honorary Research Fellowship of Foundation for Polish Science

Robert Huber. Head, Department of Structure Research, Max Planck Institute of Biochemistry, Martinsried, Germany

Wieland Huttner. Scientific Member and Director, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Leszek Kaczmarek. Professor, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Oleg A. Krishtal. Deputy Director of the Bogomoletz Institute of Physiology, Head of the Department of Cellular Membranology, Bogomoletz Institute of Physiology, Kiev, Ukraine

Jacques Mallet. Professor, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs, CNRS UMR 9923, Hopital de la Pitie-Salpetriere, Paris, France

Maciej J. Nałęcz. Director, Division of Basic and Engineering Sciences, UNESCO, Paris, France

Ryszard Przewłocki. Professor, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

J. Gregor Sutcliffe. Professor, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA

Anna Tramontano. Professor of Biochemistry, I Medical Faculty, University of Rome "La Sapienza", Rome, Italy

Directors' Note

2009 was the year of the tenth anniversary of the International Institute of Molecular and Cell Biology (IIMCB). The celebrations featured a session of the International Advisory Board, the official launch of the HEALTH-PROT project (financed under the 7th Framework Programme of the European Union) and lectures by the foreign partners in this project, a Polish-Ukrainian poster session of 36 posters, and the publication of the jubilee 10th edition of the report on the Institute's operations – the 2008 Annual Report.

Our tenth anniversary was, therefore, celebrated without a triumph of form over substance and with a strong focus on scientific content. We wished to emphasize our satisfaction with the success of IIMCB's initial years, while making no secret of our conviction that there is always room for improvement: and this is what we have placed at the foundation of our program for another decade. We take exceptional pride in the fact that the employees of our Institute have been awarded some of the most prestigious and financially gratifying research grants, such as the Wellcome Trust Senior Research Fellowships, EMBO Installation Grants, Howard Hughes Medical Institute International Research Scholar Awards, grants from the National Institutes of Health, the Max Planck Institute Partner Group and Polish-Norwegian Research Funds, as well as grants received under the 5th, 6th and 7th Framework Programmes or those awarded by the Foundation for Polish Science. Among our staff we have two newly appointed full professors who began their independent scientific careers at our Institute and who are both below 40 years of age. Our papers are published in the leading foreign journals in increasing numbers, and our alumni can readily find work in excellent scientific or biomedical laboratories all around the world.

The International Institute of Molecular and Cell Biology is entering into the second decade of its operations. There have been some specific consequences of the increasing length of the IIMCB's history: a growing complexity of the Institute's structure, increasing numbers of staff, an expansion in the number of grants to be processed and managed, and increasingly large administrative burdens. As a result, we have partially re-organized the Institute and created a number of new positions so that the new organizational structure can respond to our growing needs. The Scientific Office and the Technology Transfer Unit have been set up, and the supervision of the Institute's computer network has been outsourced to an experienced IT firm.

The economic crisis has not been without impact on our operations – government funding has become increasingly scarce and, to keep our budget balanced and ensure opportunities for growth, we rely on Polish and international grants. There are some problems, however, which cannot be solved with the help of grants: modernization, renovations, major investment in equipment or further growth into expanding laboratory space.

Our Institute is slowly approaching the capacity limits of the facilities it has been occupying until now. In addition to the ten research groups (one of which operates in Dresden), we can place only two new laboratories in our building, and this will occur quite soon. In this context, the question about continued development of the Institute is taking on a new meaning in organizational terms. There are several different scenarios under review, however each of them implies a need for significant investment in infrastructure, whether this be a completely new building, an extension of the old facility or, finally, use of other laboratory facilities available at or near the Ochota Campus. A sound and sensible size limit, which still allows a correct level of administrative and scientific manageability to be maintained, is an institute which does not expand beyond 20 research groups. This is a target size which can, and certainly should, be envisaged in the long-term. Time will show whether any of the options considered now as a possibility will even have a chance of becoming reality - and the discussion over the future shape of the Institute continues. There are many problems to be solved but there are also increasing numbers of ideas how to do so. An important thing is that all the interested parties can take part in the discussion and that each voice is received with attention.

Thanks to the structural funds obtained through a shared effort, the Ochota Campus has increasingly been gaining the status of a big, unified yet diversified, scientific organism, and it has began to operate as a whole by organizing initiatives which bring together diverse institutions. One such initiative is the Biocentrum-Ochota Consortium, which consists of five Polish Academy of Science (PAN) institutes and the IIMCB, another is the Centre for Advanced Technology and Pre-Clinical Trials (CePT), which includes Biocentrum-Ochota and the three largest academic institutions in Warsaw (Warsaw University, Medical University of Warsaw and Warsaw University of Technology). The shared activities include, for example, lecture series for all doctoral students, shared projects and scientific conferences, purchases of cutting-edge research equipment, computer infrastructure for shared purpose and use, and new buildings. In our vision we envisage joint development and management of the Ochota complex – after all, this is the largest campus hosting biomedical and biotechnology institutions in this part of Europe.

Description of the Institute's Activities

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, who supervises the organization and activities of the Institute. The President of PAN nominates members of IAB and the Institute's Directors. An important link between the Institute and the President of PAN is the 2nd Department of Biological Sciences of PAN, to which the Institute belongs together with sixteen institutes of PAN.

The Organization of Research at IIMCB

Ten research groups comprise the structure of IIMCB: Department of Molecular Biology (Żylicz), Laboratory of Bioinformatics and Protein Engineering (Bujnicki), Laboratory of Structural Biology MPG/PAN (Bochtler), Laboratory of Neurodegeneration (Kuźnicki), Laboratory of Biomodelling (Filipek), 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 p53 and molecular chaperones and oncogenic activity of MDM2 and mutated forms of p53 (Żylicz's group).
2. Development and application of computer software for structural bioinformatics of proteins and nucleic acids and theoretical and experimental studies on enzymes acting on nucleic acids (protein structure prediction, evolutionary analyses, functional characterization, protein engineering) (Bujnicki's group).
3. Crystallographic structure determination of biological macromolecules (Bochtler's group).
4. Studies on the molecular basis of neurodegenerative disease (identification of mutations in Alzheimer's disease-related genes of Polish patients and functional analysis of these mutations, the search for biomarkers, and potential therapeutic targets of Alzheimer's disease) and studies of proteins implicated in the mechanisms of learning and memory and pathogenesis of Alzheimer's disease (Ca2+ sensors belonging to calmyrin family, β-catenin, cyclin-dependent kinase 5) (Kuźnicki's group).
5. Molecular modeling of structure and function (molecular switches) of proteins and their oligomerization and complexes, focusing on rhodopsin and other G protein-coupled receptors; molecular role of mutations of presenilins in neurodegenerative diseases (Filipek's group).
6. Interdependence between intracellular endocytic transport and nuclear signal transduction (Miączynska's group)
7. Molecular processes, including gene transcription, kinase-dependent cell signaling, cytoskeleton dynamics, intracellular trafficking underlying neuronal development and plasticity, and central nervous system pathologies (tuberous sclerosis, epilepsy, neurodegenerative disorders) (Jaworski's group).
8. Mechanics of the actomyosin cortex; study of cortical contractility and the role of cortical mechanics during cytokinesis and migration (Paluch's group).
9. Structural and biochemical studies of nucleic acid enzymes (Nowotny's group).
10. Biogenesis of mitochondrial proteins (Chacińska's group).

Awards, Honors and Titles

- Janusz M. Bujnicki, Professorial title by the President of Poland
- Matthias Bochtler, Professorial title by the President of Poland
- Janusz M. Bujnicki, Fellowship for Outstanding Young Scientists of the Ministry of Science and Higher Education
- Janusz M. Bujnicki, 1-class award of the Ministry of Science and Higher Education for research achievements and a book „Prediction of Protein Structures, Functions, and Interactions" published in 2008
- Jacek Jaworski's Neuron 2009 Jan 15 paper as Highlight of the Year on the FENS website
- Agnieszka Obarska-Kosińska and Elżbieta Purta, START Fellowship for young scientists for 2009 by Foundation for Polish Science (FNP). Advisor of both theses: Janusz M. Bujnicki
- Magdalena Błażejczyk and Adam Sobczak, awards for their PhD theses distinguished by the Scientific Council of the Nencki Institute. Advisor of both theses: Urszula Wojda
- Emilia Białopiotrowicz, the Mozołowski Award presented by the Polish Biochemical Society for outstanding young biochemists in 2009 for her project and presentation entitled: „Disturbances of the cell cycle in lymphocytes from Alzheimer's dementia patients". Thesis advisor Urszula Wojda
- Bożena Kuźniewska, the main award at the 5th Conference of International Natural Sciences Students in Wilno for the presentation of her MSc research on apoptosis and the cell cycle of lymphocytes in Alzheimer's disease patients. Thesis advisor Urszula Wojda

- Łukasz Świech, Young Investigator Award, IX International Polish Neuroscience Meeting/Ist Regional FENS Meeting, Warsaw, Poland

Publishing NEWSKO

Since 2000 e-bulletin NEWSKO provides the Ochota Campus community with current information on seminars, symposia, conferences, job opportunities and other essential events. NEWSKO, which has been published at the Institute for the last nine years, integrates scientists, students and medical doctors at the Ochota Campus and plays a significant role as the communication platform for all Centres of Excellence at the Ochota Campus. Currently this information is available at www.iimcb.gov.pl/seminars.php.

Computer Network

During 2009 we managed to successfully finish modernization of our network infrastructure by constructing additional internet access points. This allows users to log on to the net without the need to actually plug in.

The Institute's servers and computer cluster (Property of the Laboratory of Bioinformatics and Protein Engineering and the Laboratory of Biomodelling) were moved to a special basement location. This enabled us to integrate the most valuable technology assets of the Institute in one place, allowing us to make better use of them. To ensure an optimum working environment and keep the equipment in good condition, we installed almost all of the computers into rack cabinets and provided our equipment with a double AC unit cooling system. The server room is also protected against unauthorized access.

In 2009, a software audit was launched by an external validation firm, to establish which software was used and whether all the software was licensed. Software without valid licenses was removed from computers.

To improve the quality of network services our IT department has been outsourced. We are hoping that thanks to such measures our efficiency will be higher and our operating costs lower than before. We chose the best offer out of three possibilities on the market and, since the successful bidder - New Business Technologies - is a company with extensive experience in computer systems integration, it will hopefully meet our expectations. NBT's current task is to provide a professional IT growth strategy appropriate to our needs.

Scientific Meetings and Lectures

- Polish-Ukrainian research collaboration meeting with poster session (posters from the Institute of Molecular Biology and Genetics in Kiev and IIMCB). 14.05.2009, Warsaw, Poland
- Kick-off meeting of the HEALTH-PROT project, 7th Framework Programme European Commission, 15.05.2009, Warsaw, Poland
- International Research Symposium "10th Anniversary of the International Institute of Molecular and Cell Biology", 15.05.2009, Warsaw, Poland
- International Conference "Challenges of molecular genetic testing in Poland – the proposal for regulations", 3.06.2009, Warsaw, Poland, coorganized by IIMCB
- IIMCB Annual Report Session, 5.06.2009, Warsaw, Poland

Seminars of invited speakers

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

Jerzy Mozrzymas (Department of Biophysics, Medical Academy, Wrocław; Recipient of the Wellcome Trust International Senior Fellowship) "Modulation and plasticity of synaptic transmission in the CNS", 22.01.2009

Artur Osyczka (Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków; Recipient of the Wellcome Trust International Senior Fellowship) "Productive and unproductive electron transfers in cofactor chains of cytochrome bc1", 19.02.2009

Maria Anna Ciemerych-Litwinienko (Department of Cytology, University of Warsaw; Recipient of fellowships from L'Oreal Poland and "Polityka") "Stem cells and muscle regeneration", 7.05.2009

Agnieszka Dobrzyń (The Nencki Institute of Experimental Biology, Warsaw; Recipient of the EMBO Installation Grant) "Stearoyl-CoA desaturase – a novel control point of lipid metabolism and insulin sensitivity", 28.05.2009

Stanisław Karpiński (Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences; Recipient of the WELCOME Award of the Foundation for Polish Science) "Can plants think? Evidences for light wave-length specific signaling and cellular light memory in Arabidopsis", 29.10.2009

- **Research Symposium "10th Anniversary of the International Institute of Molecular and Cell Biology", 15.05.2009**

Wolfgang Zachariae (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) "Of daughters and sisters: control of chromosome segregation in meiosis"

Vincenza Andrisano (Department of Pharmaceutical Sciences, University of Bologna, Italy) "Multitarget directed ligands (MTDL) characterization strategy in the context of Alzheimer's disease"

Saulius Klimasauskas (Laboratory of Biological DNA Modication, Institute of Biotechnology, Vilnius, Lithuania)

*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.

"DNA methyltransferases: structural studies and redesign for novel functions"

Ted Hupp (Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, UK) "Phosphorylation switch in MDM2 control of the p53 tumour suppressor"

Anita Becker (Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany) "Novel gene defects in primary ciliary dyskinesia"

Samantha Spangler (Dept. of Neuroscience, Erasmus Medical Center, Rotterdam, The Netherlands) "Regulation of presynaptic composition and function by distinct Liprin-alpha family proteins"

Oleg A. Krishtal (Department of Cellular Membranology, Bogomoletz Institute of Physiology, Kyiv, Ukraine) "Purinergic receptors in sensory neurons"

- **Regular IIMCB seminars**

Teresa Żołądek (Institute of Biochemistry and Biophysics, Warsaw, Poland) "The influence of ubiquitination on intracellular trafficking and actin cytoskeleton organization in yeast S. cerevisiae", 8.01.2009

Axel Methner (Klinik für Neurologie, Universitätsklinikum Düsseldorf, Germany) "STIM1 in the brain: A multielectrode array approach", 26.01.2009

Agnieszka Chacińska (Institute for Biochemistry and Molecular Biology, Freiburg, Germany) "Biogenesis and turnover of mitochondrial intermembrane space proteins", 28.01.2009

Adrianna Łoniewska-Lwowska (Department of Protein Biosynthesis, IBB PAN, Warsaw, Poland) "How do the (+) stranded RNA viruses express their genes – mechanism of the subgenomic RNA synthesis", 29.01.2009

Fabiana Renzi (Department of Structural and Chemical Biology, Mount Sinai School of Medicine and New York Structural Biology Centre Cryo-Electron Microscopy Unit, USA) "Structural studies of gamma-secretase by Electron Microscopy: An intra-membrane protease involved in Alzheimer's disease", 2.04.2009

Tomasz Węgierski (University Hospital of Freiburg, Germany) "TRP calcium channels: Cellular functions and regulatory mechanisms", 23.04. 2009

Fred Van Leuven (Experimental Genetics Group – LEGTEGG, Department of Human Genetics, Katholieke Universiteit Leuven, Belgium) "GSK3 links amyloid tau pathology in Alzheimer's disease", 28.04.2009

Zuzanna Bukowy (Institute of Biochemistry and Biophysics, Warsaw, Poland) "Role of oxidative stress in the regulation of the enzymatic activities of WRN protein", 25.06.2009

Jędrzej Szymański (German Cancer Research Center, Heidelberg, Germany) "Dynamic subcellular partitioning of the nucleolar transcription factor TIF-IA under ribotoxic stress", 16.07.2009

Maria Gorna (Department of Biochemistry, Ben Luisi group, University of Cambridge, UK) "Functional and structural studies of the Escherichia coli RNA degradosome", 31.08.2009

Tassos Perrakis (National Cancer Institute, Amsterdam, The Netherlands) "Structure-function studies of the Geminin-Cdt1 complex in DNA replication licensing", 2.09.2009

Dariusz Ekonomiuk (Computational Structural Biology Group, Department of Biochemistry, University of Zurich, Switzerland) "Several keyboards against world-wide spread viral disease", 15.09.2009

Andrew Byrd (Center for Cancer Research, National Cancer Institute, Frederick, USA) "Allosteric effects on ubiqitin ligase activity by a novel E2 binding region: Integration of NMR, crystallography, and molecular biology", 16.09.2009

S. Michał Jaźwiński (Co-Director, Center on Aging, Department of Biochemistry & Molecular Biology, New Orleans, USA) "Taking aging personally – individual paths to exceptional longevity", 8.10.2009

Oliver Griesbeck (Max Planck Institute of Neurobiology, Martinsried, Germany) "Probing the brain with fluorescent protein", 16.10.2009

Gyula Batta (Department of Biochemistry Centre of Arts, Humanities and Sciences, University of Debrecen, Hungary) "NMR structural biology research in Debrecen (an overview)", 19.11.2009

IIMCB researchers' seminars

Paweł Krawczyk (Laboratory of Molecular and Cellular Neurobiology) "Need For Speed. Aha1 Challenge", 15.01.2009

Ewa Wywiał (Laboratory of Bioinformatics and Protein Engineering) "Evolution of diversity in the membrane targeting behavior of Pleckstrin homology domains of the phospholipase C family of proteins", 22.01.2009

Sabah El Alaoui (Laboratory of Structural Biology) "Photosystem II of the cyanobacterium Thermosynechoccocus elongates: Constructions of mutants containing His-tagged (CP47;Cytb559) and point mutations", 5.02.2009

Wojciech Puławski (Laboratory of Biomodelling) "Modeling of dynamic behaviour of biological systems using the coarse-grain methods", 26.02.2009

Łukasz Bojarski (Laboratory of Neurodegeneration) "Alzheimer's disease as a lifelong 'calciumopathy", 5.03.2009

Łukasz Świech (Laboratory of Molecular and Cellular Neurobiology) "TIPs for dendritogenesis: CLIP-170 in dendritic arbor development", 12.03.2009

Łukasz Sadowski (Laboratory of Cell Biology) "On the (endo)track of PDGF", 26.03.2009

Anna Żurawska (Department of Molecular Biology) "Hsp90 inhibitor – resistant mutants", 16.04.2009

Wojciech Potrzebowski (Laboratory of Bioinformatics and Protein Engineering) "Protein structure modeling based on various experimental data", 30.04.2009

Marta Wiśniewska (Laboratory of Neurodegeneration) "A new role for b-catenin in the brain", 21.05.2009

Marcin Klejman (Department of Molecular Biology) "HSP90 – microtubules and mitosis", 18.06.2009

Jan Kosiński (Laboratory of Bioinformatics and Protein Engineering) "Dr. Kosa Farewell Seminar", 22.10.2009

Maciej Żylicz (Department of Molecular Biology) "Tumor suppressors – good and bad guys", 5.11.2009

Umesh Ghoshdastider (Laboratory of Biomodeling) "Study of bacterial cytoskeletal proteins", 12.11.2009

Marek Wojciechowski (Laboratory of Structural Biology) "Crystal structure of ThaI restriction endonuclease", 26.11.2009

Paweł Wiśniewski (Department of Molecular Biology) "Role of FasL in glioma pathogenesis", 3.12.2009

Emilia Białopiotrowicz (Laboratory of Neurodegeneration) "Genomic and proteomic instability in lymphocytes from Alzheimer's disease patients", 17.12.2009

IIMCB Annual Report Session, 5.06.2009

Maté Biro (Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden) "Mechanics and regulation of the cell cortex"

Karolina Górecka (Laboratory of Protein Structure) "Structural studies of RuvC resolvase"

Małgorzata Perycz (Laboratory of Molecular and Cellular Neurobiology) "Zipping up dendritogenesis: a role for RNA-binding proteins"

Sajid Rashid (Laboratory of Cell Biology) "APPL proteins: the novel activators of canonical Wnt/TCF signaling"

Aleksander Dębiński (Laboratory of Biomodelling) "Mechanical unfolding of proteins using molecular dynamics simulations"

Emilia Białopiotrowicz (Laboratory of Neurodegeneration) "Changes in the cell cycle regulation of human lymphocytes differentiate sporadic from familial Alzheimer's disease"

Monika Sokołowska (Laboratory of Structural Biology MPG/PAN) "Crystal structure of the ββα-Me type II restriction endonuclease Hpy99I with target DNA"

Kristian Rother (Laboratory of Bioinformatics and Protein Engineering) "The Genesilico RNA structure prediction framework"

Jakub Urbański (Department of Molecular Biology) "Modulation of Hsp90 activity in normal and cancer cells"

Matylda Macias (Laboratory of Molecular and Cellular Neurobiology) "mTOR kinase activity and aberrant neurotransmission"

Julia Roensch (Laboratory of Cell Cortex Mechanics MPG/PAN in Dresden) "Role of cortical tension in bleb growth"

Marta Olchowik (Laboratory of Cell Biology) "A novel compartment APPLied in endocytosis"

Marcin Pawłowski (Laboratory of Bioinformatics and Protein Engineering) "Novel approaches to predict and refine theoretical models of protein structure, to predict their quality and to utilize them in crystallography"

Marek Wojciechowski (Laboratory of Structural Biology MPG/PAN) "The structure of ThaI restriction endonuclease"

Wojciech Michowski (Laboratory of Neurodegeneration) "Role of zinc-binding protein CHP-1 in cell response to stress conditions".

Doctoral Degrees in 2009

Michal Gajda, PhD thesis: „Automated protein structure prediction by the FRankenstein approach". Thesis advisor: J.M. Bujnicki; Warsaw, 12.05.2009, Institute of Biochemistry and Biophysics PAN

Marcin Pawłowski, PhD thesis: „Novel approaches to predict and refine theoretical models of protein structure, to predict their quality and to utilize them in Molecular Replacement". Thesis advisor: J.M. Bujnicki; Warsaw, 9.06.2009, Institute of Biochemistry and Biophysics PAN

Joanna Sasin-Kurowska, PhD thesis: „Development of bioinformatics tools for the classification of proteins based on their tertiary structure: their application to selected family of proteins - human tyrosine phosphatases". Thesis advisor: J.M. Bujnicki; Warsaw, 14.09.2009, Nencki Institute of Experimental Biology PAN

Jan Kosiński, PhD thesis: „Dissecting molecular basis of dysfunction of protein complexes involved in DNA mismatch repair by characterization of their structure and mutual interactions, and their mechanism of action". Thesis advisor: J.M. Bujnicki; Warsaw, 22.09.2009, Institute of Biochemistry and Biophysics PAN

Magdalena Lipka, PhD thesis: „Streptogramin B lyase Vgb - structure and mechanism". Thesis advisor: M. Bochtler, Warsaw, 29.09.2009, Institute of Biochemistry and Biophysics PAN

Roman Szczepanowski, PhD thesis: „DNA base flipping by restriction endonucleases". Thesis advisor: M. Bochtler, Warsaw, 29.09.2009, Institute of Biochemistry and Biophysics PAN

Magdalena Kaus-Drobek, PhD thesis: „Restriction endonuclease MvaI". Warsaw, 5.10.2009, Thesis advisor: M. Bochtler, Institute of Biochemistry and Biophysics PAN

Wojciech Michowski, PhD thesis: „Role of the CHORD domain containing protein 1 (CHP-1) in cellular stress response". Thesis advisor: J. Kuźnicki, Warsaw, 3.11.2009, Nencki Institute of Experimental Biology PAN

Monika Sokołowska, PhD thesis: „Structural and biochemical studies of restriction endonucleases BcnI and Hpy99I". Thesis advisor: M. Bochtler, Warsaw, 10.11.2009, Institute of Biochemistry and Biophysics PAN.

Grants

7th Framework Programme

- HEALTH-PROT "Proteins in Health and Disease" (229676); 954,100 EUR; matching funds 4,099,289 PLN; 2009-2012; J. Kuźnicki
- NEURO.GSK3 "GSK-3 in neuronal plasticity and neurodegeneration: basic mechanisms and pre-clinical assessment" (223276); 280,840 EUR; matching funds 363,315 PLN 2008-2011; J. Jaworski
- SBMPs "Structural Biology of Membrane Proteins" (211800); 263,284 EUR; 2008-2012; S. Filipek

6th Framework Programme

- EURASNET "European alternative splicing network of excellence" (LSHG-CT-2005-518238); 120,000 EUR, matching funds 612,792 PLN; 2006-2010; IIMCB participation 2008-2010; J.M. Bujnicki
- MemProt "Structural studies of membrane proteases" (MTKD-CT-2006-042486); 626,800 EUR, matching funds 1,453,851 PLN; 2006-2010; M. Bochtler
- EndoTrack "Tracking the endocytic routes of polypeptidegrowth factor receptor complexes and their modulatory role on signalling" (LSHG-CT-2006-019050); 428,400 EUR; matching funds 1,011,709 PLN; 2006-2010; M. Miączyńska
- DNA Enzymes "A multidisciplinary approach to the study of DNA enzymes down to the single molecule level" (MRTN-CT-2005-019566); 254,452 EUR, matching funds 606,181 PLN; 2005-2009; J.M. Bujnicki
- PROMEMORIA "From cell-cell recognition to memory formation. New strategies for the treatment of dysfunctional plasticity, learning and memory" (LSHM-CT-2005-512012); 478,000 EUR, matching funds 1,203,600 PLN; 2005-2009; J. Kuźnicki
- EUROGENTEST "Genetic testing in Europe – Network for test development harmonization, validation and standardization of services" (LSHB-CT-2004-512148); 30,000 EUR, matching funds 60,002 PLN; 2005-2009; M. Witt

Other International Funds

- EMBO Installation Grant "Protein biogenesis and redox homeostasis in mitochondria" (1966); 150,000 EUR; 2010-2012, A. Chacińska
- Polish Norwegian Research Fund "Screening for novel functions of endocytic and autophagic proteins in the regulation of gene expression, cell growth and carcinogenesis" (PNRF-27-AI-1/07); 672,572 EUR; 2010-2011; I. Pilecka
- Polish Norwegian Research Fund "Aberrant synaptic plasticity in epilepsy" (PNRF-96-AI-1/07); 362,200 EUR; 2008-2010; J. Jaworski
- 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; 2007-2012; M. Nowotny
- NIH Grant "High-accuracy protein models derived from lower resolution data" subcontract (430-46-22 B) within a collaborative grant coordinated by A. Kloczkowski, Iowa State University, USA; 60,000 USD; 2007-2010; J.M. Bujnicki
- Howard Hughes Medical Institute, International Research Scholars "Signaling from endosomes to the nucleus: The role of APPL proteins and their interacting partners"; 500,000 USD; 2006-2010; M. Miączyńska
- The MPI-CBG/IIMCB Partner Group at the IIMCB "Biochemical and microscopial characterization of APPL-positive endosomes; 109,000 EUR; 2006-2010; M. Miączyńska
- Wellcome Trust International Senior Research Fellowship "Communication between intracellular organelles in trafficking and signalling: The role of APPL proteins" (076469); 4,315,706 PLN; 2005-2010; M. Miączyńska
- NIH Grant "Kinetoplastid SL RNA biogenesis", subcontract (2301 G EN541) within a collaborative grant coordinated by D.A. Campbell, University of California, USA; 100,440 USD; 2004-2009; J.M. Bujnicki
- Utrecht University fellowships for five PhD students (M. Witt's lab, IIMCB and Institute of Human Genetics PAN, Poznań; M. Żylicz's lab, IIMCB; A. Lipkowski's lab, Center for Experimental and Clinical Medicine, PAN, Warsaw; L. Kaczmarek's lab, Nencki Institute PAN, Warsaw); 10,000 EUR annually from 2004 to 2009
- The Max Planck Society (MPG) – the Polish Academy of Sciences (PAN) MPI-CBG Junior Research Group Program – Laboratory of Structural Biology MPG/PAN; 1,500,000 EUR, 2001-2010; M. Bochtler

Structural Funds

- POIG 1.1.2 Programme TEAM "Modeling of RNA and protein-RNA complexes: from sequence to structure to function"; 2,200,000; 2010-2014; J.M. Bujnicki
- POIG 1.1.2 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
- POKL 8.2.1 "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

- POIG 1.1.2 Programme WELCOME "Biogenesis and turnover of mitochondria intermembrane space proteins" (WELCOME/2009/1); 5,940,670 PLN; 2009-2014; A. Chacińska
- POIG 2.2.3 "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
- POIG 2.2.2 "Centre of Pre-clinical Research and Technology (CePT)" (POIG.02.02.00-14-024/08-00); 14,625,545 PLN; 2008–2013; J. Kuźnicki

Ministerial Research Grants

- "Mechanism of oncogenic activities of mutated *TP53*" (NN 302621838); 600,000 PLN; 2010-2013; A. Żylicz
- "Biochemical and structural studies of lentiviral reverse transcriptases" (NN301439738); 599,800 PLN; 2010-2013; M. Nowotny
- "Experimental characterization of hMTcap1 and hMTcap2 - last missing enzymes taking part in biosynthesis of the cap structure on human mRNA" (NN301425338); 500,000 PLN; 2010-2013; J.M. Bujnicki
- "Identifiction of the genetic program activated by Lef1/b-catenin complex in mature neurons" 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" (NN401 585738), 150,000 PLN; 2010-2011; K. Kamińska
- "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; 2009-2012; I. Pilecka
- 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
- Polish-German Special Grant „Development and implementation of methods for improving protein's crystals quality by engineering of protein-protein contacts"; 940,000 PLN; 2008-2011; J.M. Bujnicki
- "Modulation of activity of transcription factors involved in tumorigenesis, by MDM2 and other E3 ubiquitin ligases" (NN301 032534); 750,000 PLN; 2008-2011; M. Żylicz
- "Structural and biochemical studies of restriction enzymes specific for pseudopalindromic sequences" (NN301 029534); 344,400 PLN; 2008-2011; M. Bochtler
- "Functional characterization of Exonuclease G - the role in the apoptosis and diabetes" (NN401 061535); 290,400 PLN; 2008-2011; I. Cymerman
- Polish-German Special Grant "Relationship between dysregulated calcium homeostasis and synaptic pathology in Alzheimer's disease as a target for therapy" (P-N/001/2006); 1,050,000 PLN; 2007-2010; J. Kuźnicki
- Polish-Spanish Special Grant "Computer prediction and simulation of RNA tertiary structure formation" (HISZPANIA/152/2006); 553,600 PLN; 2007-2010; J.M. Bujnicki
- "Experimental characterization of the complete set of RNA methyltransferases in the model organisms and identification of their counterparts in sequenced genomes" (NN301239633); 460,000 PLN; 2007-2010; J.M. Bujnicki
- "Investigation of the mechanisms regulating expression of calmyrin2, a novel EF-hand Ca^{2+}-binding protein, and elucidation of its role in Ca^{2+}-signal transduction in physiology and in death of neurons" (N30110932/3854); 303,000 PLN; 2007-2010; U. Wojda
- "Role of dendritic mRNA transport and local protein synthesis in development of dendritic arbor of neurons" (N N301 314733); 300,000 PLN; 2007-2010; J. Jaworski
- "Investigations of activation of GPCRs by theoretical methods" (N N301 203833); 205,000 PLN; 2007-2010; S. Filipek
- "Proteins S-nitrosylation and Cdk5 kinase-dependent phosphorylation. Proteomic studies of synaptosomal fractions from transgenic mice - Alzheimer's disease models" (NN301254333); 70,000 PLN; 2007-2010; A. Szybinska (subcontractor). Coordinator: M. Dadlez, IBB
- "Role of mTOR-regulated proteins in development of dendritic tree of hippocampal and cortical neurons" (2 P04A 015 30); 220,800 PLN; 2006-2009; J. Jaworski
- Polish-German Special Grant "The role of cell cortex contractility in the establishment and positioning of the cleavage furrow", (JRGP/37/2005) The Max Planck Society (MPG) – the Polish Academy of Sciences (PAN) MPICBG Junior Research Program – Laboratory of Cortex Movement and Cell Division MGP/PAN; 3,024,200 PLN; 2006-2009; E. Paluch

Ministerial Habilitation Grants

- "Variation of restriction enzymes sequence specificities by combination of different methods of bioinformatics and protein engineering" (N30110031/3043); 278,000 PLN; 2006-2009; K. Skowronek

Ministerial Doctoral Grants

- "Automated creation and implementation of data flow schemes between bioinformatics tools" (NN301297337); 49,680 PLN; 2009-2011; J.M. Bujnicki/J. Orłowski
- "Structural and biochemical characterization of two restriction enzymes: BcnI recognizing asymmetric DNA and AvaII cutting RNA-DNA hybrids" (NN301028934); 30,600 PLN; 2008-2009; M. Bochtler/M. Sokołowska
- "MvaI restriction-modification system: different ways to recognize the same DNA sequence" (N N301 030634); 31,080 PLN; 2008-2009; M. Bochtler/M. Kaus-Drobek

- "Structural, evolutionary and functional classification of methyltransferases" (N30110532/3599); 49,000 PLN; 2007-2009; J. M Bujnicki/K. Tkaczuk
- "Modification of the substrate specificity of Bsp6I restriction endonuclease with novel methods of directed evolution" (N30204532/3598); 50,020 PLN; 2007-2009; J.M. Bujnicki/S. Pawlak
- "A novel method for assessment of global credibility and local correctness of protein structure models" (N30110632/3600); 36,600 PLN; 2007-2009; J.M. Bujnicki/ M. Pawłowski
- "Studies of agonist and antagonist binding modes in opioid receptors" (NN401140133); 50,000 PLN; 2007-2009; S. Filipek/M. Koliński

Ministerial Commissioned Grants

- "Ageing of the Polish population – medical, psychological, sociological and economic aspects" (PBZ-MEiN-9/2/2006); 12,178,420 PLN; 2007-2010; Director: P. Błędowski, coordinator M. Mossakowska
- "Novel computer programs for homology modelling and fold recognition of RNA" (PBZ/MNiSW/07/2006/04 POLPOSTDOC III); 240,000 PLN; 2007-2010; M. Boniecki
- "Structural studies of restriction endonucleases generating unusual cleavage patterns" (PBZ/MEiN/01/2006/24 POLPOSTDOC II); 160,000 PLN; 2007-2009; H. Czapińska
- "Advanced molecular methods in haematology. Development and implementation of standarized research procedures for minimal residual disease, posttransplantation chimerism and marker translocations" (PBZ-KBN-120/P05/2004); 3,027,500 PLN; 2005-2009; 13 groups in Poland; Director: M. Witt

Ministerial Commissioned Grants coordinated by other institutions

- Three tasks within a commissioned grant (PBZ-MNiI-2/1/2005) "Application of contemporary functional genomics and bioinformatics to characterize and develop models of biological processes of medical and agricultural interest": 1) Modeling of protein structures and their complexes, 2) A database of systems for DNA repair and degradation, 3) Experimental analyses of DNA repair proteins; 340,000PLN; 2006-2009; J.M. Bujnicki
- "From patterns of gene expression and regulatory motifs towards prediction and modeling of global gene expression in brain physiology and pathology", Director: B. Kamińska-Kaczmarek from Nencki Institute of Experimental Biology; within the commissioned grant: „Application of functional genomics and bioinformatics for characterization and modeling of biological processes of critical importance for medicine and agriculture" (3/0-PBZMNiI-2/1/2005); 375,000 PLN; 2006-2009; J. Jaworski

Ministerial Research-and-Development Grant

- "New tools for analysis and manipulations of nucleic acids: restriction enzymes acting on RNA and DNA-RNA hybrids" (R1200202); 1,000,000 PLN; 2007-2010; JM. Bujnicki

Other Research Grants

- ERA-NET NEURON, 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" (1/2010); 1,085,875 PLN; 2010-2013; J. Jaworski
- Project for International Scientific Cooperation (PICS) „How RNA methyltransferases may control mRNA expression" 2010-2012; B. Lapeyre/J.M. Bujnicki
- Scientific Network "Visualization of biomedical phenomena" – BIOWIZJA coordinated by the Institute of Fundamental Technological Research (63/E-89/BWSN-0142/2008); 200,000 PLN; 2008-2009; J. Bujnicki and M. Nowotny
- Grant from Foundation for Polish Science (Homing Programme) "Post-translational modifications and nuclear functions of endosomal APPL proteins" (HOM/ ed2007/126); 80,000 PLN; 2007-2009; I. Pilecka
- Scientific Network organized by Institute of Pharmacology PAN – "Looking for systemic targets of potential neurotrophic drugs" (26/E-40/BWSN-0023/2008); 54,680 PLN; 2006-2009; J. Kuźnicki/M. Wiśniewska

Publications resulting from grants (not affiliated to IIMCB research groups)

- Polosak J, Roszkowska-Gancarz M, Kurylowicz A, Owczarz M, Dobosz P, Mossakowska M, Szybinska A, Puzianowska-Kuznicka M. Decreased expression and the Lys751Gln polymorphism of the XPD gene are associated with extreme longevity. Biogerontology. 2009 Aug 26. [Epub ahead of print]
- Zietkiewicz E, Wojda A, Witt M. Cytogenetic perspective of ageing and longevity in men and women. J Appl Genet. 2009; 50(3):261-73

Other Consortial Projects Coordinated at IIMCB

Molecular haematology project

2009 was the year of completion of the project entitled *Advanced molecular methods in hematology. Development and implementation of standards of minimal residual disease, post-transplant chimerism and marker translocations analysis*, commissioned at the government minister level. The project, with an overall budget of about 3 mln PLN, linked elements of pediatric and adult molecular hematology related to basic research and practical applications. The project consortium was composed of the major Polish centers of molecular hematology in Poznań, Warsaw, Kraków, Lublin and Zabrze, which created the core of a reference laboratory network active in this area in Poland. Hematological disorders selected for the study by the consortium were: pediatric acute lymphoblastic leukemia (ALL), pediatric and adult acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and non-Hodgkin lymphomas (NHL), chosen as model disorders. Post-transplant chimerism and minimal residual disease (MRD) were analyzed in connection to the disorders listed above. Specific computer databases for ALL and AML were created, linking the available clinical and molecular data of patients recruited to the program. During the course of this project a multicenter collection of relevant biological material was created. The program was initiated at IIMCB and was conducted by Prof. Michał Witt and coordinated by Dr. Małgorzata Mossakowska. The major outcome of the project, in addition to the organizational results and research findings, was a monographic book "Molecular hematology", edited by M. Witt, T. Szczepański and M. Dawidowska, which was published in 2009 by OWN Poznań and widely acclaimed by Polish hematologists, biotechnologists and laboratory diagnosticians.

PolSenior project

The IIMCB was one of the major initiators of multidisciplinary projects on ageing and is currently a coordinator of the ministerial commissioned project PolSenior entitled *"Medical, psychological and economical aspects of ageing in Poland"*. The country's largest project in this area of research, with a budget of over 12 million PLN, aims to conduct an interdisciplinary study of various ageing-related problems encompassing diverse research disciplines concerning the ageing process in the Polish society. Specialists involved range from sociologists, psychologists, economists and demographers to geriatricians, cardiologists, nephrologists, neurologists, epidemiologists and molecular biologists from research centers of Białystok, Bydgoszcz, Gdańsk, Lublin, Lódź, Katowice, Kraków, Poznań, Szczecin, Wrocław, and Warszawa. About 40 research groups are engaged in the project. Till the end of 2009 about 3,500 (out of 6,000 planned) respondents have been visited by nurses, the questionnaires have been filed out and morphological and biochemical analysis performed. Institute also provides the whole consortium with samples of biological material and with database constructed specifically for the project. Biological material from 2 900 respondents is deposited at the IIMCB. The genomic DNA has been isolated from 600 samples.

The project is being conducted by the International Institute of Molecular and Cell Biology in Warsaw with Prof. Piotr Błędowski (President of the Polish Gerontological Society) as a head of the project and Dr. Małgorzata Mossakowska as a coordinator.

Cooperation with Other Institutions

Ochota Biocenter

In 2008 the Ochota Biocenter consortium was created, consisting of six institutes belonging to or connected with the Polish Academy of Sciences: International Institute of Molecular and Cell Biology, Nencki Institute of Experimental Biology, Mossakowski Medical Research Center, Institute of Biochemistry and Biophysics, Nałęcz Institute of Biocybernetics and Biomedical Engineering, Institute of Fundamental Technological Research. The aim for this multilateral agreement was a concentration of research potential of all consortiants enabling applications for and realizations of large-scale projects in the field of biomedicine and bioengineering. The Ochota Biocenter is governed by the Board of Directors with the President rotating among all six directors every 6 months. The financial basis of activities of Ochota Biocenter is currently formed by a number of grants (e.g. for technology transfer, for bioinformatic infrastructure, for biomedical visualization).

Centre of Pre-clinical Research and Technology (CePT)

Together with other research institutes, IIMCB implements a Project „Centre of Pre-clinical Research and Technology" (CePT), supported by Structural Funds, Operational Programme Innovative Economy (OP IE), Priority 2 R&D Infrastructure, Measure 2.2, Support for development of research infrastructure of scientific entities. The aim of the project is to create the biggest biomedical research centre in Poland within the Ochota Campus. Ten research institutions engaged in CePT (six above mentioned Ochota Biocenter institutes together with the Institute of High Pressure Physics, Warsaw University of Technology , Warsaw University and Medical University of Warsaw) will carry out basic and pre-clinical research in fields such as structural and functional analysis of proteins, physicochemistry, nanotechnology of biomaterials, molecular biotechnology, pathophysiology and physiology, oncology, genomics, neurobiology and neurological diseases. Research activities of the Ochota Research Campus will be supported by a large-scale data processing and computation centre with acknowledged international achievements. CePT infrastructure will consist of 10 interrelated core facilities integrating the main research activities of the Ochota Research Campus. Achieving CePT objectives requires construction of two buildings and utilizing other buildings financed from extramural sources. Expenditures for purchasing equipment for 10 core facilities are estimated at 250 million PLN, bringing the total estimated project cost to almost 400 million PLN. The investment will be executed in stages between 2009 and 2013. Within CePT IIMCB will create the Centre of Function of Protein Structure Analysis. The project is coordinated by the Medical University of Warsaw (WUM).

Max Planck Society

The Laboratory of Cell Cortex Mechanics MPG/PAN headed by Dr. Ewa Paluch, a twin lab of Matthias Bochtler's MPG/PAN laboratory operating at IIMCB since 2001, continued its activities (started February 2006). The equipment and running costs for the lab, including personnel, are covered from the Polish funds, but the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG), being a host for this laboratory, is responsible for local operational costs, maintenance, and administrative support. Dr. Ewa Paluch's group focuses on biochemical and physical mechanisms of cell shape and deformations. The research is concentrated on movements of the actomyosin cortex, the involvement of spontaneous cortical ruptures and flows in cell division in particular.

Dr. Marta Miączyńska, a leader of Laboratory of Cell Biology at the International Institute of Molecular and Cell Biology (IIMCB) in Warsaw, is heading a Partner Group of the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden. The MPICBG/IIMCB Partner Group was established based on a 3-years contract between the two institutions in January 2006, further extended until December 2010. From the side of MPI-CBG, Prof. Marino Zerial (Managing Director of MPI-CBG) is the cooperation partner and a scientific mentor of the Partner Group. Dr. Miączyńska has been working in the group of Prof. Zerial in Dresden as a senior postdoctoral fellow in years 2001-2005, before her return to Poland in April 2005. The scientific project of the Partner Group dealing with the characterization of APPL-positive endosomes is a continuation of the work that Dr. Miączyńska carried out in the laboratory of Prof. Zerial in Dresden.

Foreign scientists at IIMCB

- **Frank King**, MSc (USA) – PhD student in the Department of Molecular Biology, 1999-2001; graduated in Oct., 2001
- **Sanne Mikkelsen**, MSc (Denmark) – involved in Polish Centenarians Program PolStu99, then in the Laboratory of Neurodegeneration, 1999-2001
- **Sophie Chiron** (France) – senior technican at Cell and DNA Bank, Polish Centenarians Program PolStu99, 1999-2000
- **Matthias Bochtler**, PhD (Germany) – Head of the Laboratory of Structural Biology MPG/PAN Junior Research Group, 2000-present
- **Sergey Odintsov**, MSc (Belarus) – SMM's PhD student in the Laboratory of Structural Biology MPG/PAN, 2001-2004
- **Ahmad Noor Jalili**, MD (Iran) – PhD student in the Laboratory of Molecular Neurology, 2002-2003

- **Tiziana Cacciamani**, PhD (Italy) – Post-doctoral fellow in the Laboratory of Department of Molecular Biology, 2003 (2 months)
- **Gang Zhao**, PhD (China) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2003-2004
- **Michael Kreutz**, PhD (Germany) – Senior researcher in the Laboratory of Neurodegeneration, 2004 (2 months)
- **Rashid Sajid**, PhD (Pakistan) – Post-doctoral fellow in the Laboratory of Cell Biology, 2006-2009
- **Kristian Rother**, PhD (Germany/Finland) – Post-doctoral fellow in the Laboratory of Bioinformatics and Protein Engineering, 2006-2009
- **Neli Kachamakova**, PhD (Bulgaria) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2006-2007
- **Laura Lopez Munoz**, BSc (Spain) – MSc student in the Laboratory of Bioinformatics and Protein Engineering 2006-2007 (one semester)
- **Tran Cat Dong**, PhD (Vietnam) – Post-doctoral fellow in the Laboratory of Neurodegeneration, 2007 (2 months)
- **Nguyen Trong Hung**, MD (Vietnam) – PhD student in the Laboratory of Neurodegeneration, 2007 (1 month)
- **Dario Piano**, PhD (Italy) – expert involved in EU grant MEMPROT, the Laboratory of Structure Biology, 2007-2009
- **Elisa Tomat**, PhD (Italy) – visiting researcher (Dept. of Chemistry, MIT) in the Laboratory of Molecular and Cellular Neurobiology, July 7-25, 2008
- **Sabah El Alaoui**, PhD (Spain) – expert involved in EU grant – MEMPROT, the Laboratory of Structure Biology, 2008–2009
- **Umesh Ghoshdastider**, MSc (India) – PhD student involved in EU grant SBMPs within 7th FP"Marie Curie Networks for Initial Training", the Laboratory of Biomodelling, since Aug. 2009
- **Dragos Trinca**, PhD (Romania) – experienced researcher involved in EU grant SBMPs within 7th FP"Marie Curie Networks for Initial Training", the Laboratory of Biomodelling, 2009 (3 months)
- **Jean-Philippe Borges**, PhD (France) – researcher involved in EU grant MEMPROT, the Laboratory of Structure Biology, since Jan. 2010
- **Inmaculada Mora Espi**, MSc student (Spain) – volunteer in the Laboratory of Mitochondrial Biogenesis, since March 2010

Lab Leaders Competitions

The principles of organization of the Institute are distinct and differ from other research institutes in Poland: 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 five years; the progress of research is assessed by the International Advisory Board after the first 3 years and then every 2 years, and the professor's contract may be either terminated or extended. 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. Each competition is advertised in internationally visible sources, both hard copy and electronic.

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). Short-listed 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 is supposed to come with the binding decision based on this recommendation.

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

[1)]these competitions fulfilled the MPG/PAN agreement

[2)]no result

[3)]the winner did not accept the offer

Proteins in Health and Disease HEALTH-PROT

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

Objectives

HEALTH-PROT project is targeted at continuation and expansion of activities initiated as the 5th FP Centre of Excellence in Molecular Bio-Medicine (CEMBM). In the past as the Centre of Excellence we developed an advanced methodology of analysis of complex protein structures with the use of cell and molecular biology techniques, biochemical methods, crystallographic analysis and computer modelling. Our goal is **to become a top protein studies Centre in the region** by unlocking the potential of all our research groups. This will be achieved mainly by **twinning each of the Institute's groups with European groups** leading in the field **(first objective)**, through joint research activities, organization of workshops and conferences and participation in consortia within FP7. We plan to **create the place for experienced researchers to conduct research at the highest level (second objective)**, and for junior researchers to obtain the best possible mentoring and a degree based on the top-flight theses. We also intend to **reach an ultimate critical mass** by completing the organisation of IIMCB's structure **(third objective)**. We aspire to be **more innovative towards applications** in medicine and biotechnology **(fourth objective)**. In parallel, we will **popularize science and raise social awareness** of the benefits of modern biology and biotechnology **(fifth objective)**. We will leverage the activities of the existing professional popularization science unit and use the available electronic media to disseminate knowledge on protein research. Ultimately, a reinforced S&T potential of our research groups will allow us to become **more visible and attractive as a collaborating partner in the European Research Area**, for both academia and industry.

Twinning partners and their projects

Matthias Bochtler, Laboratory of Structural Biology, IIMCB and **Ruedi Allemann**, University of Cardiff, UK. ***The structure and function of proteases and endonucleases with relevance to human medicine.***

Janusz M. Bujnicki, Laboratory of Bioinformatics and Protein Engineering, IIMCB and **Saulius Klimasauskas**, Laboratory of Biological DNA Modification, Institute of Biotechnology, Vilnius, Lithuania. ***Enzymes acting on nucleic acids.***

Sławomir Filipek, Biomodelling Laboratory, IIMCB and **Vicenza Andrisano**, Department of Pharmaceutical Sciences, University of Bologna, Italy. ***Understanding of beta-amyloid formation in Alzheimer's Disease.***

Jacek Jaworski, Laboratory of Molecular and Cellular Neurobiology, IIMCB and **Casper Hoogenraad**, Erasmus MC, Rotterdam, The Netherlands. ***mTOR dependent microtubule dynamics in shaping dendritic arbor in physiological and pathological brain development.***

Jacek Kuźnicki, Laboratory of Neurodegeneration, IIMCB and **Jochen Herms**, Ludwig-Maximilians-University of Munich, Centre for Neuropathology, Germany. ***Relationship between deregulated calcium homeostasis and synaptic pathology in Alzheimer's disease as a target for therapy.***

Marta Miączyńska, Laboratory of Cell Biology, IIMCB and **Harald Stenmark**, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway. ***Endosomal proteins in regulation of cell signaling and proliferation.***

Marcin Nowotny, Laboratory of Protein Structure, IIMCB and **Roland Marquet**, Retroviruses and RNA viruses laboratory, RNA Architecture and Reactivity Unit, Université Louis Pasteur, CNRS, Strasbourg, France. ***Structural and biochemical studies of reverse transcriptases.***

Michał Witt, Ciliary Proteins Function Project, IIMCB and **Heimut Omran**, Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany. ***Role of ciliary proteins in pathogenesis of cilia-related disorders.***

Maciej Żylicz, Department of Molecular Biology, IIMCB and **Ted Hupp**, Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, UK. ***Molecular chaperones in cell transformation.***

Project progress (April 2009 – March 2010)

Increasing scientific expertise through twinning

Within this workpackage 6 experienced researchers from twining laboratories came to IIMCB to give lectures and to discuss cooperation within the project at it's kick-off meeting: **Wolfgang Zachariae** from the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany; **Saulius Klimasauskas** from the Laboratory of Biological DNA Modification at the Institute of Biotechnology in Vilnius, Lithuania; **Vincenza Andrisano** from the Department of Pharmaceutical Sciences, University

of Bologna, Italy; **Samantha Spangler** from the Department of Neuroscience at the Erasmus Medical Center in Rotterdam, The Netherlands; **Anita Becker** from the Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany; and **Ted Hupp** from the Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, United Kingdom.

Other visits to IIMCB

Olivier Griesbeck, Max Planck Institute of Neurobiology, Martinsried, Germany

Visits of IIMCB experienced researchers at the twinning institutions

A. Żylicz and **M. Żylicz** visited **Ted Hupp** at the Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, UK

Jacek Kuźnicki visited **Jochen Herms** in the Centre for Neuropathology at the Ludwig-Maximilians-University of Munich, Germany.

Jacek Jaworski visited **Casper Hoogenraad** at the Erasmus Medical Centre in Rotterdam, The Netherlands.

Beata Pyrzyńska and **Ewelina Szymańska** visited **Marino Zerial's** laboratory at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany

Aleksandra Szybińska visited **Jochen Herms's** laboratory in the Centre for Neuropathology at the Ludwig-Maximilians-University of Munich, Germany

Ewa Ziętkiewicz visited **Heimut Omran's** laboratory at the Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany

Honorata Czapińska visited **Ruedi Allemann's** laboratory at the University of Cardiff, Great Britain

Expanding research capacity

To increase research capacity at IIMCB 7 **experienced scientists** selected through an open international competition were employed. Additionally, an Equipment Specialist was employed to support scientists in specialized equipment usage.

Honorata Czapińska, Laboratory of Structural Biology, head Matthias Bochtler.

In the protease field, a post-doc uses TAP-tagged strains to isolate 26S proteasome subunits or subcomplexes. In the endonuclease field, we focus on Spo11 and its interaction partners, which play a key role in meiotic recombination, and on the RAG1/2 recombination endonucleases, which are responsible for somatic recombination.

Marcin Pawłowski, Laboratory of Bioinformatics and Protein Engineering, head Janusz M. Bujnicki.

A postdoctoral researcher is involved in the development of computer software dedicated to prediction, modeling, and analysis of protein and nucleic acid structures. In particular, he develops computer programs for modeling of protein-RNA complexes and uses these programs to build models of RNPs implicated in medically important processes.

Marta Wiśniewska and **Tomasz Węgierski**, Laboratory of Neurodegeneration, head Jacek Kuźnicki.

Newly hired post-docs are involved in the analysis of changes in calcium homeostasis and synaptic function in cells with mutations causing familial Alzheimer disease (FAD).

Elżbieta Nowak, Laboratory of Protein Structure, head Marcin Nowotny.

The postdoctoral researcher works on purification and crystallization of reverse transcriptase from S. cerevisiae LTR retrotransposon Ty3. We plan to determine crystal structures of Ty3 RT in complex with RNA/DNA hybrids. Unlike HIV and HBV RT, Ty3 enzyme is monomeric and there is no structural information available about the mode of substrate binding for monomeric reverse transcriptases.

Zuzanna Bukowy, Ciliary Proteins Function Project, head Michal Witt.

The postdoctoral researcher performs in vitro culturing of airway epithelial cells and detail microscopic analysis of de- and reciliation of these cells. Slow-motion video microscopic analysis of ciliary beat pattern of various PCD patients is being performed.

Paweł Wiśniewski, Department of Molecular Biology, head Maciej Żylicz.

A new postdoctoral researcher works on modulation of activity of transcription factors involved in tumorigenesis, by MDM2 and other E3 ubiquitin ligases. Screening for such transcription factors which react with MDM2 but are not ubiquitylated and degraded by proteasome is being performed aimed at elucidation if MDM2 exerts its chaperone activity on some transcription factors.

Roman Szczepanowski, equipment specialist assists newly employed postdoctoral researchers in scientific and technical matters related to the usage of highly specialized research equipment.

Organization of scientific events

During the first project's year organization of the following events has been initiated:

- Workshop **„Biology of cancer"** 12-13.06.2010, Warsaw, 30 participants; organizers Alicja Żylicz and Maciej Żylicz
- **ECS meeting** 6-9.09.2010; **workshop „Calcium and toolkit"**, Warsaw, 300 participants; organizer Jacek Kuźnicki

- Workshop **„Proteins: structures, folding, and interactions''**, 27-30.08.2010, Warsaw, around 60 participants; organizers Janusz M. Bujnicki, Matthias Bochtler and Marcin Nowotny
- Workshop **„Mechanisms of cytoskeleton dynamics and intracellular trafficking"** 21-24.10.2010, Warsaw, around 60 participants; organizers Marta Miączyńska and Jacek Jaworski

Participation in international events

- **Jan Kosiński**, ISMB/ECCB, Sweden
  Poster: Kosinski J, Bujnicki JM "Prediction of causative effects of disease related mutations at the molecular level by integrated bioinformatics analyses"
- **Marcin Pawłowski**, ISMB/ECCB, Sweden
  Poster: Pawlowski M, Bujnicki JM "Importance of local model quality in molecular replacement method"
- **Janusz M. Bujnicki**, ISMB/ECCB, Sweden
  Poster: Musielak M, Puton T, Rother K, Bujnicki JM "ModeRNA: a new software tool for comparative modeling of RNA 3D structures"
- **Marcin Nowotny**, European Crystallographic Meeting, Turkey
  Poster: Nowotny M, Gaidamakov S, Crouch RJ, Yang W "Structural Studies of RNases H and their Complexes with RNA/DNA Hybrids"
- **Matthias Bochtler**, European Crystallographic Meeting, Turkey
- **Roman Szczepanowski**, European Crystallographic Meeting, Turkey
  Poster: Szczepanowski R, Czapinska H, Carpenter M, Zaremba M, Tamulaitis G, Siksys V, Bhagwat A, Bochtler M "DNA flipping by restriction endonucleases"
- **Honorata Czapińska**, European Crystallographic Meeting, Turkey
  Poster: Czapińska H, Sokolowska M, Bochtler M „ββα-Me restriction endonuclease Hpy99I in complex with target DNA"
- **Grzegorz Chojnowsk**i, European Crystallographic Meeting, Istambul
  Poster: Chojnowski G, Bochtler M „DIBER: protein, DNA, or both?"
- **Łukasz Świech**, 22nd Biennial Meeting of the International Society for Neurochemistry (ISN), Korea
  Poster: Świech L, Blazejczyk M, Dortland, Hoogenraad, Jaworski J "In search for the missing link: CLIP-170 and mTOR kinase in dendritic arbor development"
- **Anna Toruń**, EMBO Endocytosis 2009, Greece
  Poster: Toruń A, Banach-Orłowska M, Rashid S, Pilecka I, Pyrzyńska B, Miączyńska M „APPL proteins as components and regulators of histone deacetylase-containing complexes"
- **Marta Miączyńska**, EMBO Conference Series on "Membrane Dynamics in Endocytosis", Greece
  Oral presentation: "APPL proteins: endosomal adaptors as regulators of transcription"
- **Michał Witt**, **Ewa Ziętkiewicz**, Keystone Symposium: (G1) "Cilia, Signaling and Human Disease", USA
  Poster: Zietkiewicz E, Nitka B, Rutkiewicz E, Skrzypczak U, Voelkel K, Witt M "Genetic background of primary ciliary dyskinesia in Polish patients – search for mutations in candidate genes".

Promotion

Promotional actions of the HEALTH-PROT started already at the beginning of the project and continued throughout the first year of project implementation. In May 2009 the project was initiated with a kick-off meeting, held in Warsaw, which was attended by officials from the Ministry of Science and Higher Education, National Contact Point, Polish Academy of Sciences, HEALTH-PROT twinning partners and IIMCB International Advisory Board members.

In June the project website was launched which was subsequently expanded with the HEALTH-PROT events and activities. It can be viewed at: *http://www.iimcb.gov.pl/hp* for further details.

The project was also promoted by its poster which was presented during various international and domestic events. The poster is displayed on the cover of the Annual Report.

In October 2009 Joanna Lilpop representing the IIMCB's Centre for Innovative Bioscience Education visited the National Science Learning Centre, University of York, UK. The following actions were delivered: (i) oral presentation of the HEALTH-PROT – its aims, research groups and scientific objectives. (ii) HEALTH-PROT poster exhibited at the poster session "Sharing science as a process of discovery", (iii) leaflets left in the area open for visitors at the NSLC.

In March 2010 IIMCB organized open days of the HEALTH-PROT project. 32 secondary school students from whole Poland listened to presentations delivered by postdoctoral researchers employed in the project and by the project's managers. Then they visited the Institute.

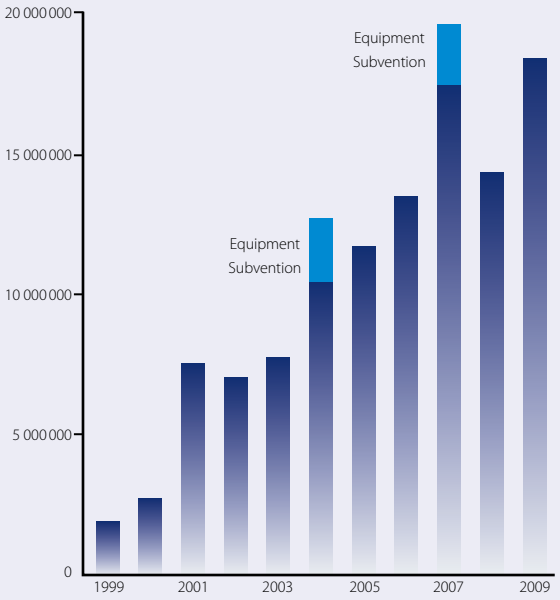
In March 2010 Urszula Białek-Wyrzykowska participated in the conference „Week of Innovative Regions in Europe" (WIRE2010), Granada, Spain co-organized by The Spanish Presidency to the EU Council and by the European Commission. She presented there a case study on the project.

Management

The project's progress was reviewed during 7 monthly meetings of Task Leaders and Project Managers. In November 2009 a meeting of Workpackage Leaders, and Task Leaders and Project Managers was organized to summarize the project achievements during the first six months of it's implementation (the report available at: *http://www.iimcb.gov.pl/hp/management.html*).

Diversity of Funding IIMCB'2009

Annual income (in PLN)



Sources of Funding	amounts in PLN	amounts in EUR[1]
Statutory Subvention	4 248 326	1 034 109
Budgetary Subvention	1 066 000	259 481
Individual Domestic Grants	3 564 040	867 543
*Consortial Domestic Grants	2 334 860	568 341
Structural Funds	467 700	113 845
**Supplementary Financial Support of Foreign Grants	1 144 746	278 649
**Foreign Grants	5 231 986	1 273 547
Equipment Subvention	523 345	127 390
Total	**18 581 003**	**4 522 906**

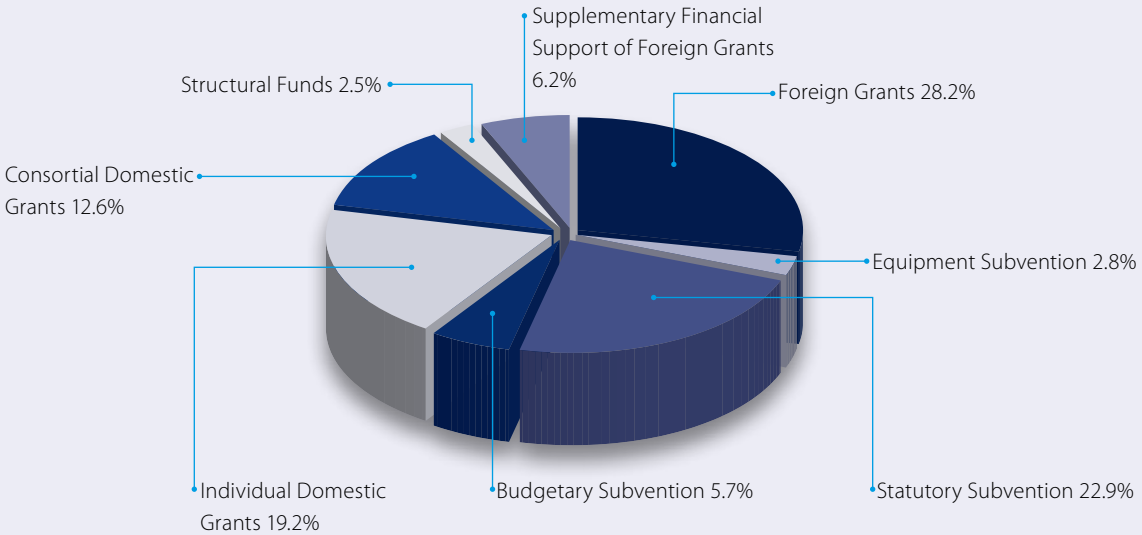
[1] 1EUR – 4,1082 @ 31st Dec' 2009

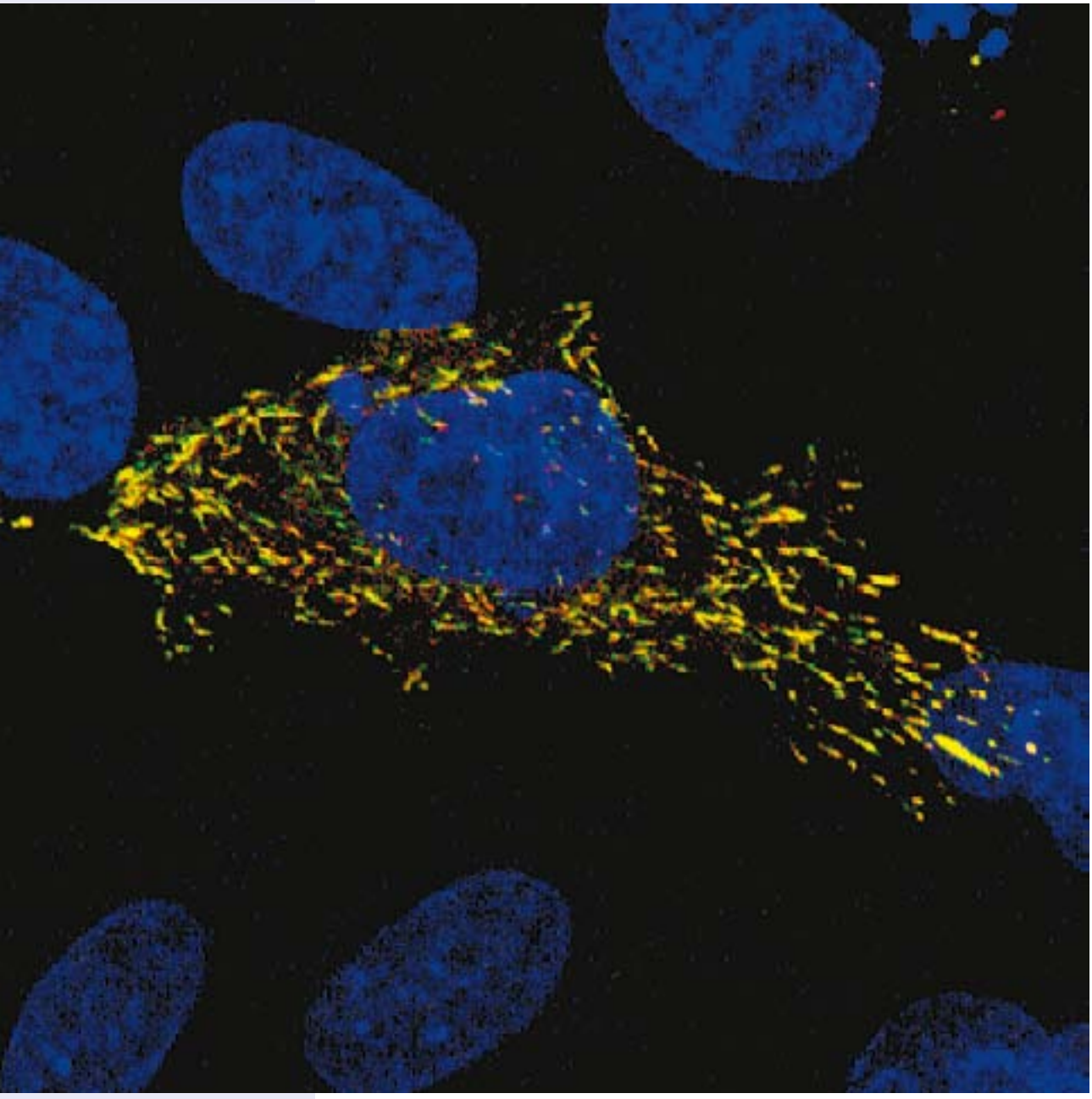
* Funds for Partners of the PolSenior Project excluded

** Funds for 2010 and 2011 of HEALTH-PROT Project excluded

Profit & loss statement

	amounts in PLN
A. net revenue on sales and equivalents	16 703 147
B. operational activity costs:	17 510 100
Depreciation (equipment)	1 255 739
Research materials	3 770 976
Utilities	412 030
Services	2 782 295
Fees and taxes	706 743
Salaries and wages	5 684 805
Social and health insurance	1 331 823
Other operational expenses, in this:	1 565 689
business trips	712 872
property insurance	18 020
fellowships	815 325
others	19 472
C. other operational income (subventions)	806 953
D. other operational expenses:	10 358
E. financial income (interest):	107 034
F. financial expenses (other):	47 468
Profit on business activity (A-B+C-D+E-F)	**49 208**





GFP-CLIP170 microtubule plus-tip interacting protein (green) colocalizes with HA-Lis1 (red) in HeLa cells. DNA was stained with DAPI (blue) (author: Marcin Klejman).

Department of **Molecular Biology**

Lab Leader:

Maciej Żylicz, PhD, Professor

Vice Head:

Alicja Żylicz, PhD, Professor

Research Associates:

Marcin Klejman, PhD

Dawid Walerych, PhD

Paweł Wiśniewski, PhD

Junior Researchers:

Marta Małuszek, MSc

Zuzanna Szymańska, MSc

Zuzanna Tracz, MSc

Jakub Urbański, MSc

Milena Wiech, MSc

Aleksandra Dudek, MSc student

Natalia Sikorska, MSc student

Secretary:

Grażyna Orleańska, MSc

Technician:

Wanda Gocal

Maciej Żylicz
PhD, Professor

DEGREES
- Professor, 1992
- DSc. Habil. in molecular biology, Institute of Biochemistry and Biophysics PAN, Warsaw, Poland, 1986
- PhD in biochemistry, Medical University of Gdańsk, Poland, 1979
- MSc in physics, University of Gdańsk, Poland, 1977 (student of physics and biology)

POST-DOCTORAL TRAINING

1982-1984 University of Utah, Department of Cellular, Viral and Molecular Biology, Salt Lake City, UT, USA and Stanford University, Department of Biochemistry, USA

1979-1981 University of Gdańsk, Department of Biochemistry, Gdańsk

PROFESSIONAL EMPLOYMENT

since 2005 President, Executive Director of the Foundation For Polish Science (FNP)

since 1999 Head of the Department of Molecular Biology, IIMCB

1994-1999 Head of the Department of Molecular and Cellular Biology, Faculty of Biotechnology, University of Gdańsk

1991-1994 Head of the Department of Molecular Biology, University of Gdańsk

1993-1994 Visiting Professor, University of Utah, Medical Center, Institute of Oncology, Salt Lake City, UT, USA

1990-1993 Vice President, University of Gdańsk

1988-1991 Associate Professor, Department of Molecular Biology, University of Gdańsk

1981-1988 Assistant Professor, Department of Biochemistry, University of Gdańsk

OTHER PROFESSIONAL ACTIVITIES

2000-2004 Chair of Biology, Earth Sciences and Environmental Protection Commission of the State Committee for Scientific Research (Poland)

since 2008 - Panel Chair, Molecular and Structural Biology and Biochemistry (LS1), ERC, Brussels

MEMBERSHIP IN SCIENTIFIC SOCIETIES, ORGANIZATIONS AND PANELS
- Full Member of the Polish Academy of Sciences
- Member of the Polish Academy of Arts and Sciences
- Member of the Academia Europaea
- Member of the American Society of Biochemistry and Molecular Biology
- Member of EMBO
- Member of the Advisory Editorial Board of EMBO Journal, EMBO Reports (2004-2008) and IUBMB Life
- Member of EMBO Council (2004-2007)
- Member of the Selection Committee for EMBO YIP (2001-2003)
- Polish delegate to EMBC (2001-2004)
- Member of the State Committee for Scientific Research (1997-2004)
- Polish delegate to the Life Science Committee of ESF (2003-2005)
- Member of the Selection Committee for the special DFG programmes (2001-2005)

HONORS, PRIZES, AWARDS
1. Officer's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland (2008)
2. Doctor Honoris Causa of University of Wrocław, 2007
3. Prime Minister Award for Scientific Achievements, 2002
4. "L. Marchlewski" Award from the Biochemistry and Biophysics Committee PAN, 2001
5. Award from the Foundation for Polish Science (FNP) in biological/medical sciences, 1999
6. Awards from the Polish Biochemical Society for the best biochemistry work performed in Polish laboratories, 1996, 2007
7. Award from the Ministry of Education, 1994
8. "Heweliusz" Prize for the Scientific Achievements, awarded by the President of Gdańsk, 1993
9. Award from the Polish Academy of Sciences, 1990
10. Individual Award from the Polish Academy of Sciences for Scientific Achievements, 1986

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.

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 PNAS and more than 25 in J Biol Chem. These papers were cited around 5 500 times. The Hirsch parameter around H=40.

Selected publications

- **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. (2009) 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
- Spiechowicz M, **Zylicz A**, **Bieganowski P**, Kuznicki J, Filipek A. Hsp70 is a new target of Sgt1- an interaction modulated by S100A6A. Biochem Biophys Res Commun, 2007; 357:1148-53
- **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 GC-content 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 is mainly focused on activities of molecular chaperones in mammalian cells, including cell transformation (for review, see Zylicz et al., EMBO J, 2001). Using highly purified recombinant human proteins, we previously identified intermediate reactions leading to the assembly of molecular chaperone complexes with the wild-type or mutant p53 tumor suppressor protein (King et al., EMBO J, 2001). More recently, we demonstrated that the Hsp90 molecular chaperone is required for binding of wild-type p53 to the promoter sequences under a physiological temperature of 37°C and that this chaperoning activity is ATP-dependent (Walerych et al., J Biol Chem, 2004). We provided *in vivo* evidence that Hsp90 and Hsp70 chaperone machines are required for proper folding of wild-type p53, its specific binding to chromatin, and transcription of p53-dependent genes (Walerych et al., Oncogene, 2009). p53 as an unstable protein *in vitro* likely requires stabilizing factors to act as a tumor suppressor *in vivo*. We have shown that in human cells transfected with wild-type p53, Hsp90, and Hsp70, molecular chaperones maintain the p53 native conformation under heat-shock conditions (42°C) and assist p53 refolding at 37°C during the recovery from heat-shock. We also demonstrated that the interaction of wild-type p53 with the *WAF1* promoter in cells is sensitive to Hsp70 and Hsp90 inhibition at 37°C and further decreased upon heat-shock. The influence of chaperones on p53 binding to the *WAF1* promoter sequence has been confirmed *in vitro* using highly purified proteins. Hsp90 stabilizes p53 binding to the promoter sequence at 37°C, whereas under heat-shock conditions, the requirement for the Hsp70-Hsp40 system and its cooperation with Hsp90 increases. The Hop co-chaperone additionally stimulates these reactions. Interestingly, the combined Hsp90 and Hsp70-Hsp40 allows for a limited *in vitro* restoration of the DNA binding activity by the p53 oncogenic variant R249S and affects its conformation in cells. Our results indicate for the first time that, especially under stress conditions, not only Hsp90 but also Hsp70 is required for the chaperoning of wild-type and R249S p53.

In collaboration with Prof. Jacek Jassem, a clinician at the Medical University of Gdańsk, we previously demonstrated that MDM2 overexpression was a new independent factor of adverse prognosis in non-small cell lung cancer (Dworakowska et al., Lung Cancer 2004). We recently discovered that MDM2, in addition to its E3-ubiquitin ligase activity, also possesses molecular chaperone activity. We demonstrated that a MDM2 mutant protein 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). In collaboration with the Prof. Ted Hupp laboratory, we developed a system for the analysis of the molecular chaperone function of MDM2 toward its target proteins (e.g., transcription factor E2F1; Stevens et al.,FEBS J, 2008).

The MDM2 oncoprotein plays multiple regulatory roles in the control of p53-dependent gene expression. A picture of MDM2 is emerging in which structurally discrete but interdependent functional domains are linked through changes in conformation. The domain structure includes the following: (i) a hydrophobic pocket at the N-terminus of MDM2 that is involved in both its transrepressor and E3-ubiqutin ligase functions, (ii) a central acid domain that recognizes a ubiquitination signal in the core DNA binding domain of p53, and (iii) a C-terminal C2H2C4 RING finger domain that is required for E2 enzyme-binding and ATP-dependent molecular chaperone activity. In collaboration with the Prof. Kathryn Ball laboratory (University of Edinburgh), we showed that the binding affinity of MDM2's hydrophobic pocket can be regulated through the RING finger domain and that increases in pocket affinity are 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 $I_{0.5}$ for p53 transrepression. An allosteric model for regulation of the hydrophobic pocket is supported by differences in protein conformation and pocket accessibility between wild-type and RING domain mutant MDM2 proteins. Additionally, the data demonstrate that the complex relationship between different domains of MDM2 can impact the efficacy of anticancer drugs directed toward its hydrophobic pocket (Wawrzynow et al., J Biol Chem, 2009).

Interferon regulatory factor-1 (IRF-1), the founding member of the interferon regulatory factor family, is a transcription factor that regulates a diverse range of target genes during

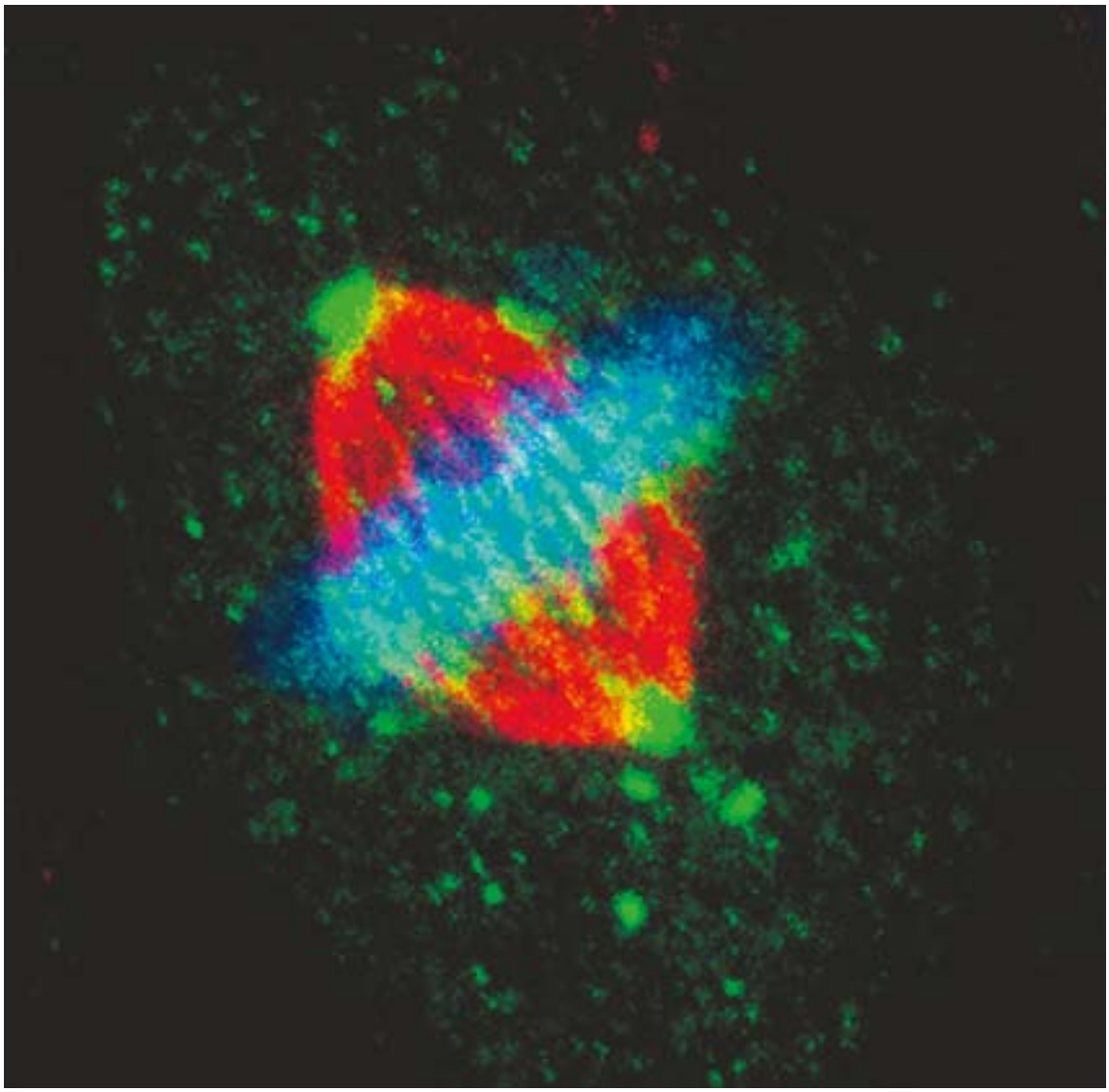


Fig. 1. GFP-CLIP170 microtubule plus-tip interacting protein (green) colocalizes with HA-Lis1 (red) in HeLa cells. DNA was stained with DAPI (blue) (author: Marcin Klejman).

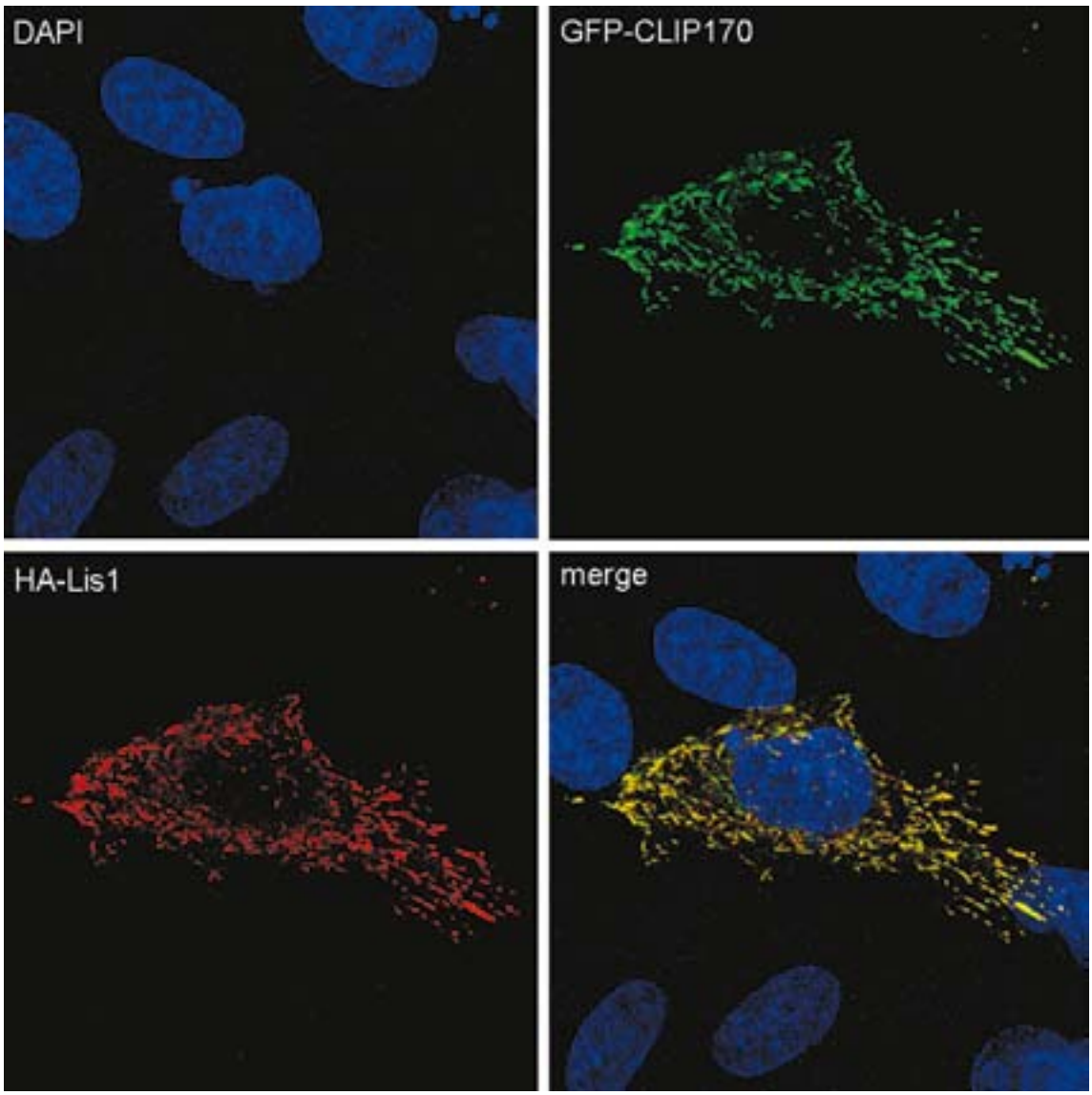
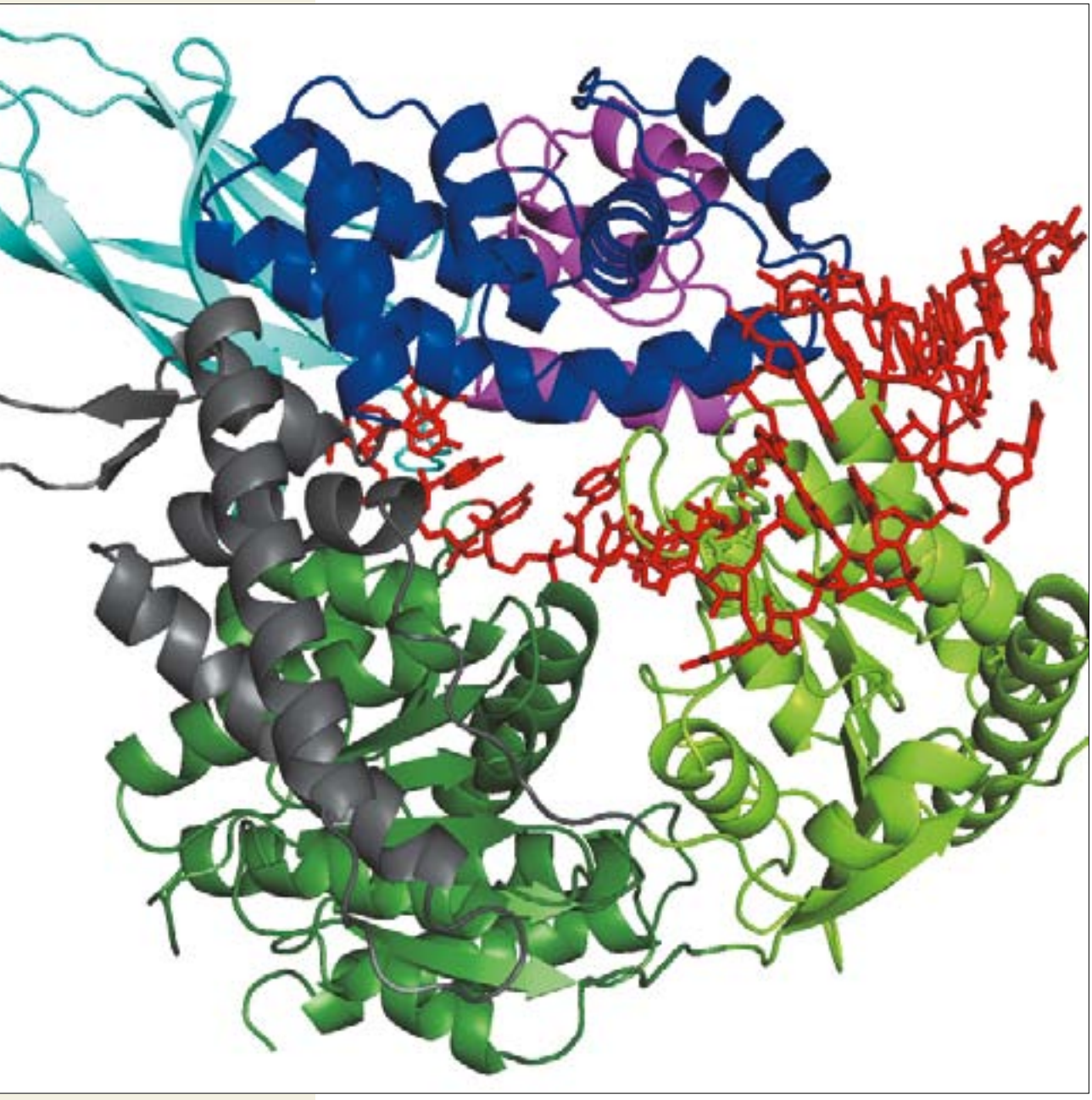


Fig. 2. GFP-CLIP170 microtubule plus-tip interacting protein (green) colocalizes with HA-Lis1 (red) in HeLa cells. DNA was stained with DAPI (blue) (author: Marcin Klejman).

the response to stimuli, such as pathogen infection, DNA damage, and hypoxia. Additionally, the loss of IRF-1 can cooperate with c-Ha-*ras* in cellular transformation; it becomes up-regulated in cells that bear oncogenic lesions, and deletions of *IRF-1* are associated with the development of gastric and esophageal tumors and some leukemias. In collaboration with the Prof. Kathryn Ball laboratory, we provided evidence linking IRF-1 to the Hsp70 family and Hsp90, the core components of the molecular chaperone machinery. Narayan et al. (J Biol Chem, 2009) demonstrated a requirement for the C-terminal multifunctional-1 (Mf1; amino acids 301-325) domain of IRF-1 in the recruitment of Hsp70 proteins. Consequently, Hsp70 was shown to recruit Hsp90, which together impact turnover, localization, and activity of IRF-1. The data highlight a novel IRF-1 interaction that contributes to its activation pathway, suggesting that the molecular chaperones are key components of a regulatory network that maintains IRF-1 tumor suppressor function.

In the search for novel Hsp90-interacting proteins, we identified a family of Hsp90-interacting proteins—NudC (nuclear distribution protein C homolog). The NudC family shares a CS domain with other Hsp90-interacting proteins, including p23 and Sgt1. We showed that NudC proteins differ in their ability to bind Hsp90. NudC and NudCD3, but not NudCD1, interact with Hsp90 in an ATP-dependent manner. Hsp90 binding is not necessary for NudC stability; therefore, NudC appears to be a co-chaperone or an auxiliary protein. We are currently studying the mechanism of NudC interactions with Hsp90 using both purified proteins and cell culture-based assays. NudC is known to interact and modulate the function of mitotic Polo-like kinase 1. Moreover, it regulates trafficking of cargo on microtubules via the dynein/dynactin complex. Hsp90 also interacts with Plk1 and was implicated in cell-cycle regulation. To investigate the interplay between NudC, Plk1, and NudC, we designed several NudC mutants defective in Hsp90 binding. We speculate that the Hsp90-NudC interaction is necessary to properly control mitosis by Plk1.



Structural model of the N-terminal „catalytically active" Sec63 module in the spliceosomal RNA helicase Brr2 from S. cerevisiae. The protein is shown in the „cartoon" rendering, with different domains shown in distinct colors. The tentative position of RNA is shown in red. This model has been constructed by Jerzy Orłowski and Janusz M. Bujnicki based on the crystal structure of homologous „inactive" C-terminal Sec63 module of Brr2 solved in the group of Markus Wahl (Freie Universität Berlin). This research has been published in Mol Cell. 2009 Aug 28;35(4):454-66, „Common design principles in the spliceosomal RNA helicase Brr2 and in the Hel308 DNA helicase", Pena V, Jovin SM, Fabrizio P, Orlowski J, Bujnicki JM, Lührmann R, Wahl MC.

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Michał Boniecki, PhD,

Marcin Pawłowski, PhD,

Ewa Wywiał, PhD

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Undergraduate Students:

Piotr Wojciechowski, BSc; Sebastian Opałczyński, BSc; Natalia Borkowska, Magdalena Mika, Tomasz Stępniewski, Marta Bogdał, Albert Bogdanowicz, Anna Górska, Małgorzata Habich, Piotr Iwaniuk, Krzysztof Suchoński, Katarzyna Ziółkowska

Office Manager:

Agnieszka Faliszewska, MSc

Computer Administrators:

Jan Kogut, MSc; Tomasz Jarzynka, Łukasz Munio

Janusz Bujnicki
PhD, Professor

DEGREES

2009	The title of Professor of Biological Sciences, President of the Republic of Poland
2005	DSc. Habil. in biochemistry; Institute of Biochemistry and Biophysics PAN, Warsaw
2001	PhD in biology; University of Warsaw, Faculty of Biology
1998	MSc in microbiology; University of Warsaw, Faculty of Biology

PROFESSIONAL EXPERIENCE

since 2002	Head of the Laboratory of Bioinformatics and Protein Engineering IIMCB
since 2006	Visiting Associate Professor, Bioinformatics Laboratory at the Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
2004-2006	Assistant Professor, Bioinformatics Laboratory at the Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
2001-2002	Group Leader, Molecular Evolution Research Group, Laboratory of Bioinformatics, IIMCB
2001	Visiting Scientist, Computational Biology Branch, National Center for Biotechnology Information, NLM, NIH, Bethesda, MD, 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, MI, USA (with Dr. L.C. Lutter)

PROFESSIONAL AFFILIATIONS

- Polish Society for Bioinformatics, PTBI (founding member and vice-president, since 2007)
- Society of Bioinformatics in Northern Europe, SocBiN (board member, since 2004)
- Member of the ELIXIR committee for building the Bioinformatics Training Strategy and the committee on global collaboration
- Member of International Society for Computational Biology and RNA Society
- Series editor, Nucleic Acids and Molecular Biology (Springer Verlag, since 2009)
- Editorial Board of Nucleic Acids Research, Advances in Bioinformatics, Journal of Applied Genetics, BMC Bioinformatics, the Database Journal, Journal of Nucleic Acids

AWARDS

2009	Fellowship for Outstanding Young Scientists of the Ministry of Science and Higher Education
2009	Award of the Ministry of Science for Research Achievements (Individual work)
2008	Adam Mickiewicz University Rector Award for Research Achievements (Individual work)
2006	Award of the Prime Minister for the habilitation thesis
2006	Young Researcher Award in Structural and Evolutionary Biology of the Visegrad Group Academies of Sciences
2005	Group award of the Ministry of Health for coauthorship of series of publications regarding the biological function of protein K (head of the team: Prof. J. Ostrowski)
2003	Fellowship for Young Scientists from the Foundation for Polish Science
2002	EMBO/Howard Hughes Medical Institute Young Investigator Program award
2002	Award from the Polish Society of Genetics (the best Polish genetics-related publication in the year: Trends Biochem Sci. 2001 Jan; 26(1): 9-11)
2001	Award from the Polish Biochemical Society (the best Polish publication on nucleic acid biochemistry in the year 2000: FASEB J. 2000 Nov; 14(14): 2365-2368)

Publications in 2009

- Leski TA, Caswell CC, **Pawlowski M**, Klinke DJ, **Bujnicki JM**, Hart SJ, Lukomski S. bcl genes of Bacillus cereus group organisms: Identification, classification and application in anthrax detection and fingerprinting. Appl Environ Microbiol 2009 Nov;75(22):7163-72
- Kolmos E, Nowak M, **Werner M**, Fischer K, Schwarz G, Mathews S, Schoof H, Nagy F, **Bujnicki JM**, Davis SJ. Integrating ELF4 into the circadian system through combined structural and functional studies. HFSP J 2009 Oct;3(5):350-366
- Pena V, Jovin SM, Fabrizio P, **Orlowski J**, **Bujnicki JM**, Lührmann R, Wahl MC. Common design principles in the spliceosomal RNA helicase Brr2 and in the Hel308 DNA helicase. Mol Cell 2009 Aug 28;35(4):454-66
- Palfi Z, Jae N, Preusser C, **Kaminska KH**, **Bujnicki JM**, Lee JH, Guenzl A, Kambach C, Urlaub H, Bindereif A. SMN-assisted assembly of snRNP-specific Sm cores in Trypanosomes. Genes Dev 2009 Jul 15;23(14):1650-64.
- Zylicz-Stachula A, **Bujnicki JM**, Skowron P. Cloning and analysis of a bifunctional methyltransferase/restriction endonuclease TspGWI, the prototype of a Thermus sp. enzyme family. BMC Mol Biol 2009 May 29;10(1):52.
- **Pawlowski M**, Lasica A, Jagusztyn-Krynicka EK, **Bujnicki JM**. AAN82231 protein from pathogenic E. coli CFT073 is a close paralog of DsbB enzymes and does not belong to the DsbI family. Pol J Microbiol 2009;58(2):181-4
- **Purta E**, O'Connor M, **Bujnicki JM**, Douthwaite S. YgdE is the 2'-O-ribose methyltransferase RlmM specific for nucleotide C2498 in bacterial 23S rRNA. Mol Microbiol 2009 Jun;72(5):1147-58.
- Bauer RA, **Rother K**, Moor P, Reinert K, Steinke T, **Bujnicki JM**, Preissner R. Fast structural alignment of biomolecules using a hash table, n-grams and string descriptors. Algorithms 2009, 2(2), 692-709; doi:10.3390/a2020692
- Matsumoto Y, Oota H, Asaoka Y, Nishina H, Watanabe K, **Bujnicki JM**, Oda S, Kawamura S, Mitani H Medaka: a promising model animal for comparative population genomics. BMC Res Notes 2009, May 10;2:88
- Pierechod M, Nowak A, Saari A, **Purta E**, **Bujnicki JM**, Konieczny I. Conformation of a plasmid replication initiator protein affects its proteolysis by ClpXP system. Protein Sci 2009 Mar;18(3):637-49.
- Kennaway CK J, **Obarska-Kosinska A**, White JK, **Tuszynska I**, Cooper LP, **Bujnicki JM**, Trinick J, Dryden DTF. The structure of M.EcoKI Type I DNA methyltransferase with a DNA mimic antirestriction protein. Nucleic Acids Res 2009 Feb;37(3):762-70.
- Nakonieczna J, Kaczorowski T, **Obarska-Kosinska A**, **Bujnicki JM**. Functional analysis of the Mmel restriction-modification enzyme from a methanol utilizer Methylophilus methylotrophus: A subtype IIC enzyme related to Type I enzymes. Appl Environ Microbiol 2009 Jan;75(1):212-23.
- Majorek K, **Bujnicki JM**. Modeling of Escherichia coli Endonuclease V structure in complex with DNA. J Mol Model 2009 Feb;15(2):173-82.
- Czerwoniec A, **Dunin-Horkawicz S**, **Purta E**, **Kaminska KH**, Kasprzak J, **Bujnicki JM**, Grosjean H, **Rother K**. MODOMICS: a database of RNA modification pathways. 2008 update. Nucleic Acids Res 2009 Jan;37(Database issue):D118-21.

Other selected publications

- **Kaminska KH**, Kawai M, **Boniecki M**, Kobayashi I, **Bujnicki JM** Type II restriction endonuclease R.Hpy188I belongs to the GIY-YIG nuclease superfamily, but exhibits an unusual active site BMC Struct Biol 2008 Nov 14;8(1):48
- **Purta E**, O'Connor M, **Bujnicki JM**, Douthwaite S YccW is the m5C methyltransferase specific for 23S rRNA nucleotide 1962 J Mol Biol 2008 Nov 14;383(3):641-51
- **Pawlowski M**, **Gajda MJ**, **Matlak R**, **Bujnicki JM** MetaMQAP: a meta-server for the quality assessment of protein models BMC Bioinformatics 2008 Sep 29;9(1):403
- **Orlowski J**, **Bujnicki JM** Structural and evolutionary classification of Type II restriction enzymes based on theoretical and experimental analyses Nucleic Acids Res 2008 Jun;36(11):3552-69.
- **Feder M**, **Purta E**, **Koscinski L**, Cubrilo S, Vlahovicek G, **Bujnicki JM**, Virtual screening and experimental verification to identify potential inhibitors of the ErmC methyltransferase responsible for bacterial resistance against macrolide antibiotics ChemMedChem 2008 Feb 15;3(2):316-322.
- **Pietal M**, **Tuszynska I**, **Bujnicki JM** PROTMAP2D: visualization, comparison, and analysis of 2D maps of protein structure Bioinformatics 2007 Jun 1;23(11):1429-30.
- **Tkaczuk KL**, **Dunin-Horkawicz S**, **Purta E**, **Bujnicki JM** Structural and evolutionary bioinformatics of the SPOUT superfamily of methyltransferases BMC Bioinformatics 2007, Mar 5;8:73.
- **Orlowski, J**, **Boniecki M**, **Bujnicki JM** I-Ssp6803I: the first homing endonuclease from the PD-(D/E)XK superfamily exhibits an unusual mode of DNA recognition Bioinformatics 2007 Mar 1;23(5):527-30.
- **Cymerman IA**, **Obarska A**, **Skowronek KJ**, Lubys A, **Bujnicki JM** Identification of a new subfamily of HNH nucleases and experimental characterization of a representative member, HphI restriction endonuclease. Proteins 2006 Dec 1;65(4):867-76.
- **Dunin-Horkawicz S**, **Feder M**, **Bujnicki JM** Phylogenomic analysis of the GIY-YIG nuclease superfamily BMC Genomics 2006 Apr 28;7(1):98
- **Tkaczuk KL**, **Obarska A**, **Bujnicki JM**. Molecular phylogenetics and comparative modeling of HEN1, a methyltransferase involved in plant microRNA biogenesis. BMC Evol Biol 2006 Jan 24;6(1):6

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 in macromolecular complexes. The laboratory comprises three sections:

1. A section devoted to the development of computer software for the analysis of biological macromolecules. The bioinformatics tools include a suite of programs for protein structure prediction and analysis available via the website https://iimcb.genesilico.pl/toolkit/ (MetaServer for primary, secondary, and tertiary structure prediction, methods for template-based and de novo modeling of three-dimensional protein structures, MetaMQAP for quality assessment of protein models, FILTREST3D for discrimination of models according to their agreement with experimental data). We also develop databases of nucleic acid metabolism, including MODOMICS, a database for systems biology of RNA modification (http://iimcb.genesilico.pl/modomics/) and the REPAIRTOIRE database for systems biology of DNA repair (http://iimcb.genesilico.pl/repairtoire/).

2. A section devoted to the application of bioinformatics software to make biologically and biomedically relevant predictions. Recently published research includes analyses of various enzymes acting on nucleic acids, such as the endonuclease V enzyme. Theoretical research in this section frequently involves collaboration with other laboratories interested in obtaining a structural model of their favorite proteins and experimental testing of our predictions. Recent modeling analyses (published in 2009) include, for example, proteins involved in the spliceosome assembly (RNA helicase Brr2 and the SMN protein) and the Type I DNA methyltransferase complex.

3. A section devoted to experimental research on proteins and nucleic acids using methods of biochemistry, molecular biology, and cell biology. Three principal types of analyses are carried out by researchers from our "wet lab":

- Experimental testing of functional predictions by gene cloning, protein expression, purification, development of in vitro and in vivo functional assays, and biochemical and cellular characterization.
- Experimental testing of structural predictions by application of low-resolution structural probing methods, such as mutagenesis, chemical modification, cross-linking, mass spectrometry, circular dichroism, and limited proteolysis.
- Protein engineering to obtain enzymes with new, useful features, particularly altered substrate specificity (e.g., restriction enzymes that recognize and cut new sequences). Other protein engineering projects include attempts to design and obtain proteins with altered tertiary and quaternary structures.

The research in all three sections is tightly integrated, demonstrated by publication of articles comprising the combination of theoretical and experimental analyses (e.g., prediction and characterization of new RNA methyltransferases). In particular, protein engineering involves iterative protein structure model building, model-based experiment planning, a series of experimental analyses, and experiment-based improvement of the models and tools used for model building.

Recent highlights

Researchers from our laboratory predicted (using bioinformatic tools) and then experimentally confirmed that a gene *ygdE* in the genome of *Escherichia coli* encodes a so far uncharacterized RNA methyltransferase. In collaboration with a group in Odense headed by prof. Stephen Douthwaite (University of Southern Denmark), we have determined that YgdE is the 2'-O-ribose methyltransferase RlmM specific for nucleotide C2498 in bacterial 23S rRNA. This is the 12th RNA methyltransferase predicted by our group and confirmed experimentally by us or in collaboration with another group. Thus far we have identified and characterized two other bacterial rRNA methyltransferases RlmI (YccW) and RlmH (YbeA), six methyltransferases acting on tRNA in bacteria, archaea and eukaryota: TrmJ (YfhQ), TrmK (YqfN), MnmC (YfcK), Trm11 (Yol124c), TrmB (YggH), and TrmI, and three methyltransferases involved in the formation of a cap structure in the SL RNA in Trypanosomes.

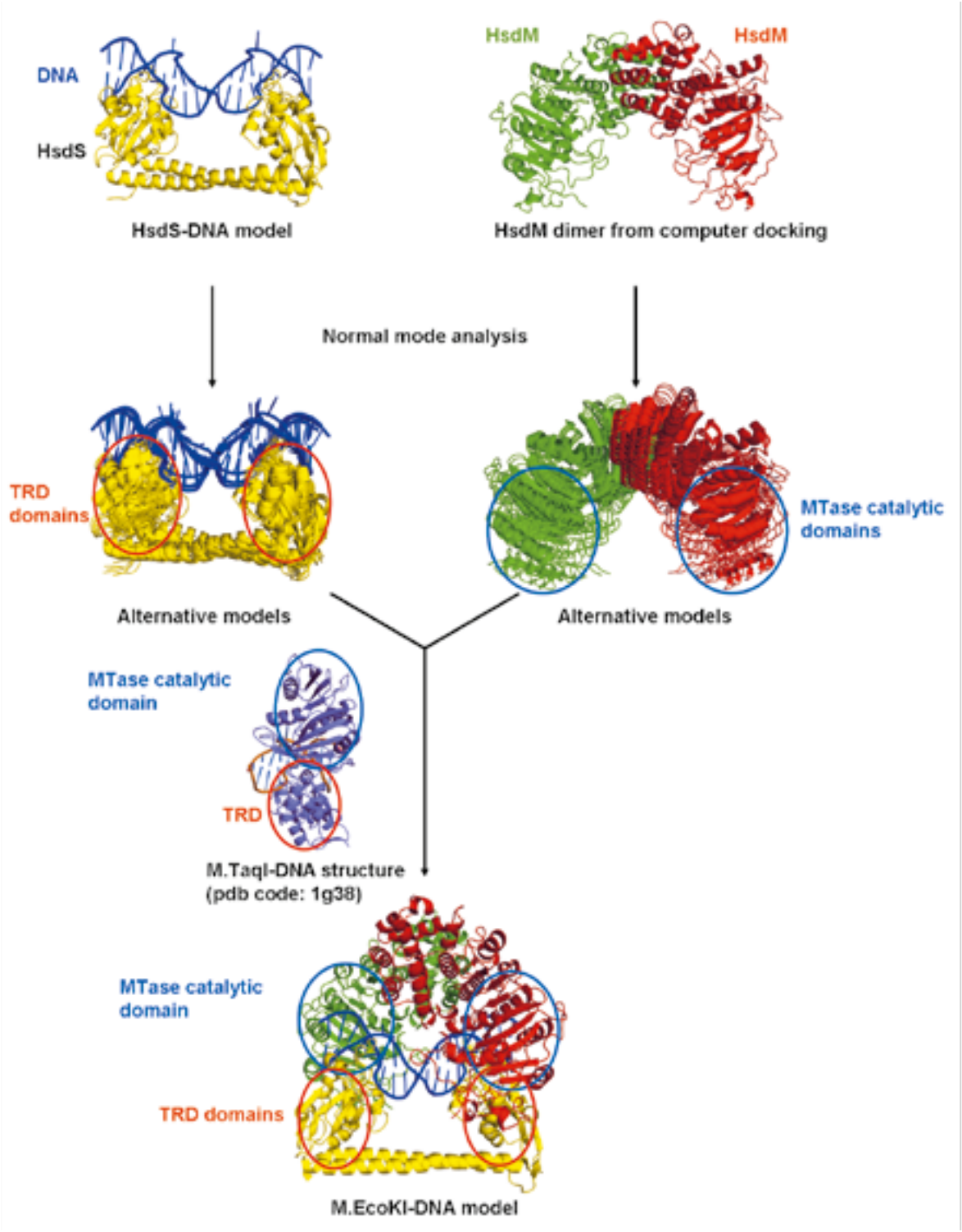
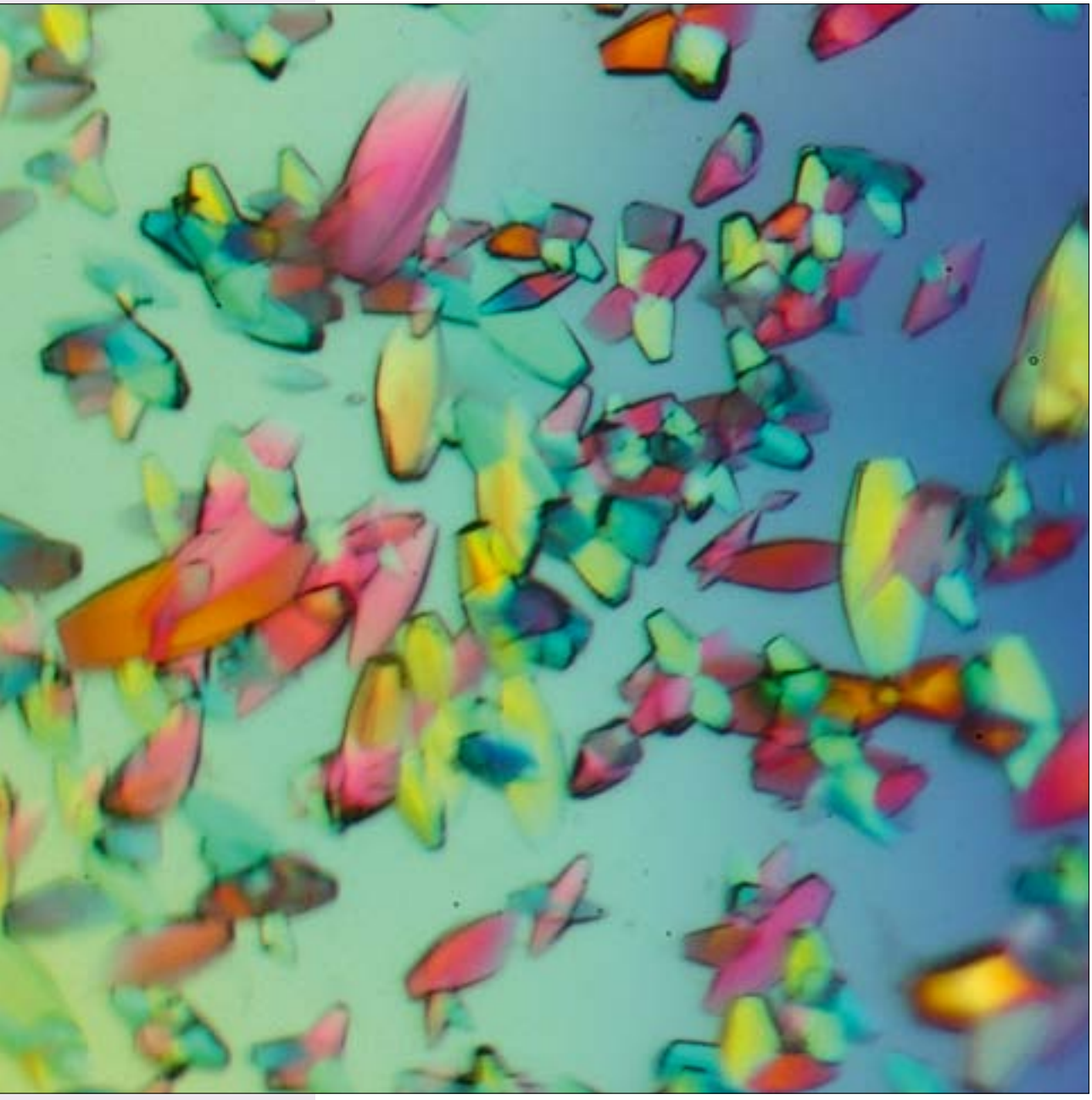


Fig. A workflow of bioinformatic methods for constructing a model of the Type I DNA methyltransferase EcoKI. A comparative model of the HsdS subunit has been constructed, and a model of HsdS-DNA complex has been built by analogy to M.TaqI methyltransferase. In parallel, a model of the HsdM subunit dimer has been constructed by docking, starting with the crystal structure of a monomer, and using data from mutagenesis experiments to infer the site of protein-protein interactions. Subsequently, HsdM and HsdS subunits have been docked to each other, taking into account global conformational changes predicted with the Normal Mode Analysis. As a result, a model of the HsdS-(HsdM)2-DNA complex has been obtained. Subsequently, a model of HsdS-(HsdM)2-ocr complex has been constructed, by replacing DNA with the ocr protein and introducing minor adjustments. The final model has been verified by fitting to electron microscopy data. The results of this analysis have been published in Nucleic Acids Res 2009 Feb;37(3):762-70. (Kennaway CK J, Obarska-Kosinska A, White JK, Tuszynska I, Cooper LP, Bujnicki JM, Trinick J, Dryden DTF „The structure of M.EcoKI Type I DNA methyltransferase with a DNA mimic antirestriction protein")



Crystals of Hpy188I-DNA complex (author: Monika Sokołowska).

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Lab Leader:

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Post-doctoral Fellows:

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Patrycja Haniewicz, MSc

Henryk Korza, MSc

Marek Wojciechowski, MSc

EU visiting experts:

Jean-Philippe Borges, PhD

Technician:

Ewa Błażewicz

MAX-PLANCK-GESELLSCHAFT

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

The equipment and running costs for the lab, including personnel, are partly provided by the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG).

Matthias Bochtler
PhD, Professor

DEGREES

- The title of Professor of Biological Sciences, President of the Republic of Poland, 2009
- DSc. Habil, Institute of Bioorganic Chemistry PAN, Poznan, Poland, 2006
- PhD in biochemistry, Technical University of Munich, Germany, 1999
- MSc in experimental physics, Munich University, Germany, 1995

RESEARCH TRAINING

1999-2000	Max Planck Institute of Biochemistry, Martinsried, Germany
1996-1999	Research Assistant, MPI of Biochemistry, Martinsried, Germany
1995-1996	Internship, the Department of Medical Microbiology, University of Regensburg, Germany
1992-1993	Guest Student, Cambridge University, United Kingdom
1990-1992	Studies in physics, Munich University, Germany

PROFESSIONAL EMPLOYMENT

Since 2007	part time Director of Structural Biology, Cardiff University, United Kingdom
Since 2001	Head of the Joint MPG-PAN Junior Group at the International Institute of Molecular and Cell Biology in Warsaw
2000	Patent training (Weickmann & Weickmann)
1999-2000	Post-doctoral Fellow at the Max Planck Institute of Biochemistry in Martinsried, Germany

HONORS, PRIZES, AWARDS

1. Pienkowski Award, 2005
2. EMBO/HHMI Young Investigator Award, 2004
3. Crystal Award, Germany, 2000
4. Crystal Award, Germany, 1998
5. Scholarship from Deutsche Studienstiftung and the Bavarian State, 1990-1992

Recent publications

Protein-DNA interactions

- 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-phox deficient chronic granulomatous disease (CGD). Hum Mutat, 2010; 31:151-158
- **Sokolowska M**, **Czapinska H**, **Bochtler M**. Crystal structure of the beta beta alpha-Me type II restriction endonuclease Hpy99I with target DNA. Nucleic Acids Res, 2009; 37:3799-3810
- Sukackaite R, Grazulis S, **Bochtler M**, Siksnys V. The recognition domain of the BpuJI restriction endonuclease in complex with cognate DNA at 1.3-A resolution. J Mol Biol, 2008; 378:1084-93
- **Szczepanowski RH**, Carpenter MA, **Czapinska H**, Zaremba M, Tamulaitis G, Siksnys V, Bhagwat AS, **Bochtler M**. Central base pair flipping and discrimination by PspGI. Nucleic Acids Res, 2008; 36:6109-17
- Tamulaitis G, Zaremba M, **Szczepanowski RH**, **Bochtler M**, Siksnys V. How PspGI, catalytic domain of EcoRII and Ecl18kI acquire specificities for different DNA targets. Nucleic Acids Res, 2008; 36:6101-8
- Tamulaitis G, Zaremba M, **Szczepanowski RH**, **Bochtler M**, Siksnys V. Nucleotide flipping by restriction enzymes analyzed by 2-aminopurine steady-state fluorescence. Nucleic Acids Res, 2007; 35:4792-9
- **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; 369:722-734
- **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(10):2219-29

Peptidases, proteases and protein degradation

- Groll M, **Bochtler M**, Brandtstetter H, Clausen T, Huber R Molecular Machines for Protein Degradation. ChemBioChem, 2005; 6:222-56
- **Szczepanowski RH**, **Filipek R**, **Bochtler M**. Crystal Structure of a Fragment of Mouse Ubiquitin-activating Enzyme. J Biol Chem, 2005; 280:22006-11
- **Odintsov SG**, **Sabala I**, Bourenkov G, Rybin V, **Bochtler M**. Staphylococcus aureus aminopeptidase S is a founding member of a new peptidase clan. J Biol Chem, 2005; Odintsov SG, Sabala I, Bourenkov G, Rybin V, Bochtler M. Substrate access to the active sites in aminopeptidase T, a representative of a new metallopeptidase clan. J Mol Biol,

2005; 354:403-12
- **Korza HJ**, **Bochtler M**. Pseudomonas aeruginosa LDcarboxypeptidase, a serine peptidase with a Ser-His-Glu triad and a nucleophilic elbow. J Biol Chem, 2005; 280:40802-12
- **Firczuk M**, Mucha A, **Bochtler M**. Crystal structures of active LytM. J Mol Biol, 2005; 354:578-90
- **Filipek R**, Potempa J, **Bochtler M**. A comparison of staphostatin B with standard mechanism serine protease inhibitors. J Biol Chem, 2005; 280:14669-74

Method development

- **Chojnowski G**, **Bochtler M**. The statistics of the highest E value. Acta Crystallogr A, 2007; 63:297-305
- **Bochtler M**, **Chojnowski G**. The highest reflection intensity in a resolution shell. Acta Crystallogr A, 2007; 63:146-155

Other

- **Chojnowski G**, Breer K, Narczyk M, Wielgus-Kutrowska B, **Czapinska H**, Hashimoto M, Hikishima S, Yokomatsu T, **Bochtler M**, Girstun A, Staroń K, Bzowska A. 1.45A 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
- **Lipka M**, **Filipek R**, **Bochtler M**. Crystal structure and mechanism of the Staphylococcus cohnii virginiamycin B lyase (Vgb). Biochemistry, 2008;47: 4257-65
- Breer K, Wielgus-Kutrowska B, Hashimoto M, Hikishima S, Yokomatsu T, **Szczepanowski RH**, **Bochtler M**, Girstun A, Staron K, Bzowska A. Thermodynamic studies of interactions of calf spleen PNP with acyclic phosphonate inhibitors. Nucleic Acids Symp Ser (Oxf), 2008; 52:663-664.

Current Research

Peptidases, proteases, and protein degradation

The most interesting peptidases of unknown structure are either very large or embedded in the membrane. The latter include several families with key roles in ontogeny. Rhomboid peptidases have pleiotropic roles in development by affecting EGF receptor signaling. γ-secretases play a physiological role in "cellular rivalry"-dependent lineage decisions by Delta/Notch signaling and a pathophysiological role in Alzheimer's disease. Although both groups of proteases are mostly known for their roles in higher eukaryotes, they are also found in prokaryotes. The first and very informative structures of bacterial rhomboid peptidases were recently solved by others. Therefore, we have concentrated our efforts on γ-secretases. Eukaryotic γ-secretase consists of a catalytically active subunit and several accessory subunits (Aph-1, Pen-2, Nicastrin). Similar to the rhomboid peptidase, the catalytic subunit of γ-secretase has orthologs in bacteria, including the PppA protein of Escherichia coli. We are currently focusing on the expression and purification of bacterial PppA proteins and are attempting to demonstrate their proteolytic activity against model substrates in vitro. To understand the basic mechanistic features of membrane aspartic peptidases, we are also studying bacterial prolipoprotein peptidases. These predicted aspartic peptidases are not phylogenetically related to γ-secretases but are nonetheless interesting as targets of the antibiotic globomycin. We are currently attempting to crystallize the protein, either alone or with inserted epitopes that are recognized by Fab fragments of available monoclonal antibodies.

Protein-DNA interactions

Two-fold symmetry is a recurrent theme in protein-DNA interactions. Classic examples are the interactions between type II restriction endonucleases and their two-fold symmetric (palindromic) target sequences. In most cases, two-fold symmetry governs sequence recognition and DNA cleavage. Exact two-fold symmetry is only possible in DNA duplexes that consist of an even number of base pairs. In duplexes that consist of an odd number of base pairs, the requirements of hydrogen bonding and two-fold symmetry conflict for the central base pair. Therefore, such sequences can at best be pseudosymmetric (pseudopalindromic). At the center, the possibilities for base recognition are limited—either the bases in this position are not read out at all, or A:T pairs (W) are distinguished from G:C pairs (S), regardless of which DNA strand contains the purine and which contains the pyrimidine base. The latter type of recognition poses difficulties for the "typical" major groove readout of the DNA base sequence. In the major groove, the hydrogen bonding patterns of a G:C and T:A are similar but differ from the patterns for C:G and A:T. The research group has systematically studied restriction endonucleases that recognize and cleave pseudopalindromic DNA sequences.

The nucleotide flippers Ecl18kI and PspGI: The related PD-(D/E)XK restriction endonucleases Ecl18kI and PspGI are specific for the sequences /CCNGG and /CCWGG, respectively. Our crystal structures of these enzymes show that both enzymes form functional dimers that extrude the central bases of their recognition sequences from the DNA and flip them into pockets of the enzymes.

"Nucleotide flips" have been observed previously, especially in the contexts of base modification and DNA repair. However, the nucleotide flips in the complexes of Ecl18kI and PspGI with DNA are unusual in several ways. First, the enzymes flip intact bases, which are not modified chemically. Second, the enzymes flip both bases of the DNA stack. Third, the void left behind by the flipped bases is not filled by DNA intercalating residues, which occurs in most other cases of nucleotide flipping, but is instead closed by "compression" of the DNA such that the base pairs that flank the flipped pairs come into almost direct contact. Ecl18kI simply "skips" the flipped bases for recognition, but PspGI distinguishes W from S pairs. A proposed mechanism that can explain this distinction without the need to identify individual bases is the hypothesis that nucleotide flipping might serve as a "test" of the strength of the hydrogen bonding interactions. According to this model, PspGI would be "strong" enough to break the two hydrogen bonds

that hold A:T/T:A ("W, weak"), but not G:C/C:G ("S, strong"), pairs together. Kinetic experiments show that base pair strength plays a role for base pair discrimination but is alone insufficient to explain the high specificity that was previously concluded for DNA repair enzymes that recognize DNA lesions.

The monomeric restriction endonucleases MvaI and BcnI: The PD-(D/E)XK restriction endonucleases MvaI (CC/WGG) and BcnI (CC/SGG) recognize similar sequences as EcI18kI and PspGI but cleave them with a different stagger. Nevertheless, MvaI and BcnI have evolved a very different strategy to deal with the asymmetry of their substrates: both enzymes bind their substrates as monomers. Each monomer has only one active site, suggesting that MvaI and BcnI must cleave the two DNA strands one after another, with an intermittent DNA rebinding event to bring the uncleaved strand of the nicked intermediate into a position proximal to the active site. Support for a nicked intermediate in the DNA cleavage reactions by the two enzymes is also provided by a comparison of the MvaI and BcnI structures with all structures in the Protein Data Bank. They are more similar to the DNA nickase MutH, a component of the mismatch repair machinery, than to any DNA restriction endonuclease of known structure.

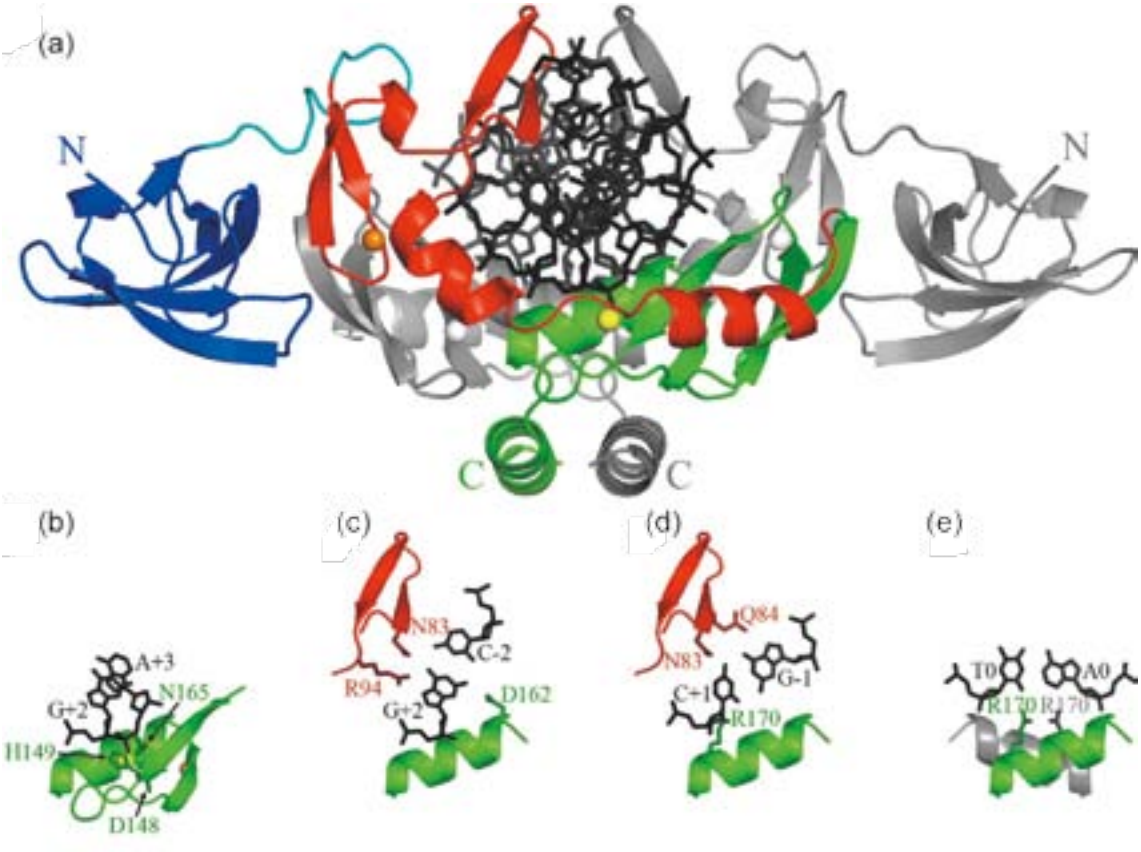


Fig. 1. Hpy99I–DNA complex. Overall structure (a) and details (b-e) of the Hpy99I-DNA complex. The figure is taken from Sokolowska et al., Nucleic Acids Res, 2009, 37:3799-3810.

The ββα-Me restriction endonuclease Hpy99I: This restriction endonuclease is specific for the sequence CGWCG/ and cuts DNA into fragments with highly unusual 5-nucleotide-long 3'-overhangs. Our recent crystal structure of Hpy99I represents the first structure of a ββα-Me restriction endonuclease and allows detailed comparisons with previously determined structures of ββα-Me endonucleases that play no role in restriction biology but are involved in nonspecific DNA degradation (such as the Serratia nuclease), homing (such as I-PpoI), or Holliday junction resolution (T4 endonuclease VII). Hpy99I distinguishes between W and S at the center of its target sequence by exclusive minor groove readout. Unlike major groove readout, this type of sequence recognition is perfectly suitable to distinguish S and W. The presence of a guanine amino group in the central minor groove position signals a G:C/C:G pair; its absence (which is verified by two Hpy99I arginines) confirms the presence of an A:T/T:A pair. Based on mutagenesis data alone, this mechanism has been previously suggested for methyltransferases. To our knowledge, our Hpy99I-DNA co-crystal structure provides its first crystallographic demonstration (Fig. 1).

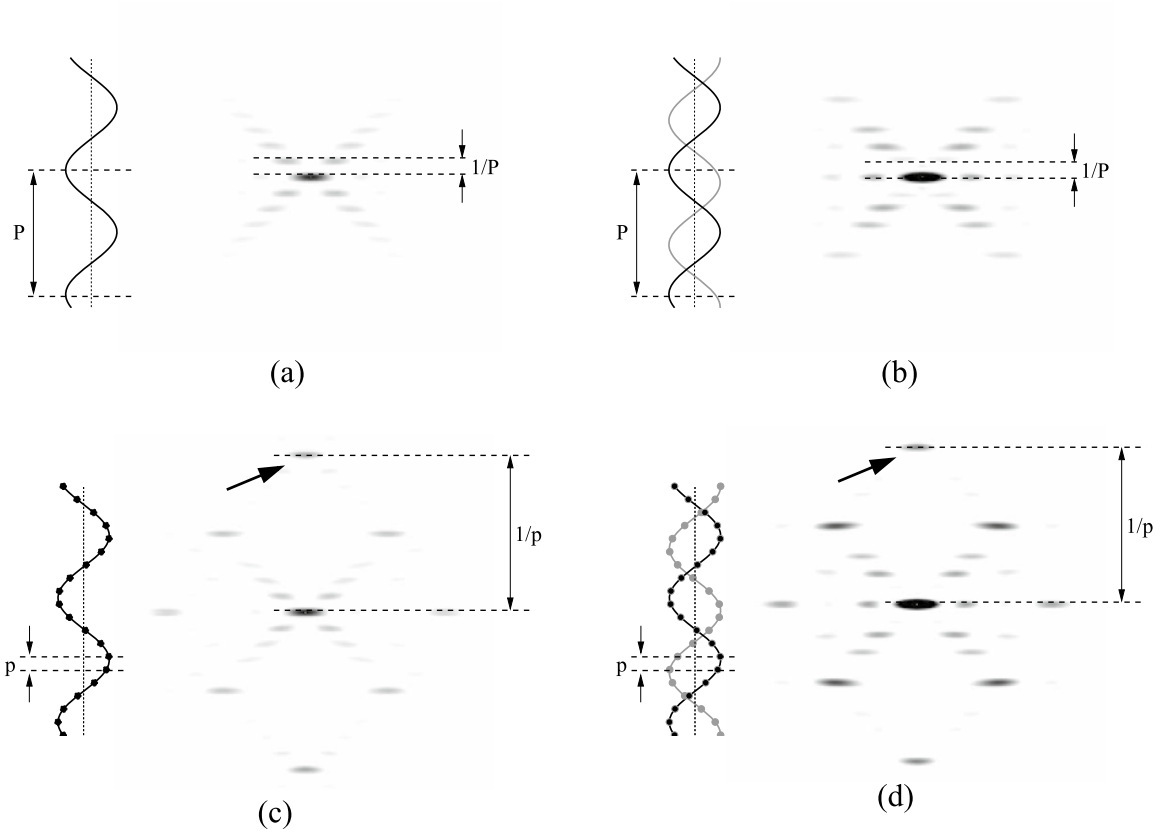


Fig. 2. Real-space and reciprocal-space representations of continuous and discontinuous helices and double helices. All calculations were performed with pitch P = 34 Å and (average) helix radius r = 7.0 Å. The axial distance between pearls in (c) and (d) was p = 3.4 Å. Layers have finite width because only two turns of the helix were used for the numerical calculations. The arrows highlight the characteristic 3.4 Å peak.

Method development

X-ray fiber diffraction photographs of proteins and nucleic acids show characteristic peaks that reflect simple repeats of these structures. In the case of B-DNA, the strong "meridional" 3.4 Å reflections are attributable to the constructive interference of scattering from base pairs at van der Waals distance (Fig. 2 and 3). For proteins, peaks of similar shape at 1.5 Å resolution and of more complex shape at lower resolution can be attributed to the presence of α-helices and β-sheets, respectively. If DNA or protein is present in three-dimensional crystals, then the characteristic fiber diffraction pattern is sampled by the reciprocal lattice, but because cell constants are typically large compared with characteristic distances in secondary structure, little information is lost by the sampling. We developed software that looks for the traces of fiber diffraction peaks in three-dimensional diffraction data. Our first tool is the program DIBER, which helps the user decide whether a dataset contains only protein, only DNA, or a mixture of both. A CCP4- and CCP4i-compatible version of DIBER is available under GNU Public Licence (http://www.iimcb.gov.pl/diber/) (Fig. 4).

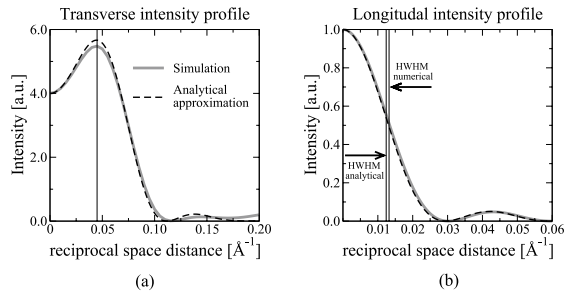


Fig. 3. Transverse (a) and longitudinal (b) intensity profile of the 3.4 Å peak of in the Fourier transform of dsDNA. Analytical (broken lines) or numerical (grey lines) calculations were done for a helix that consists of 10 base pairs.

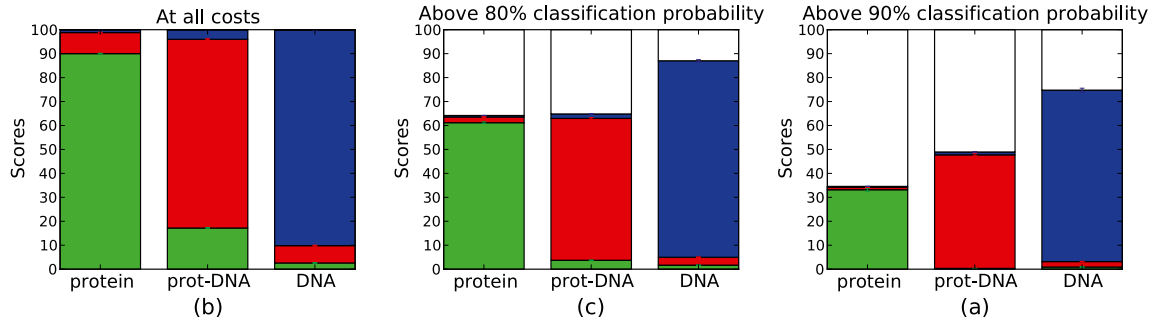
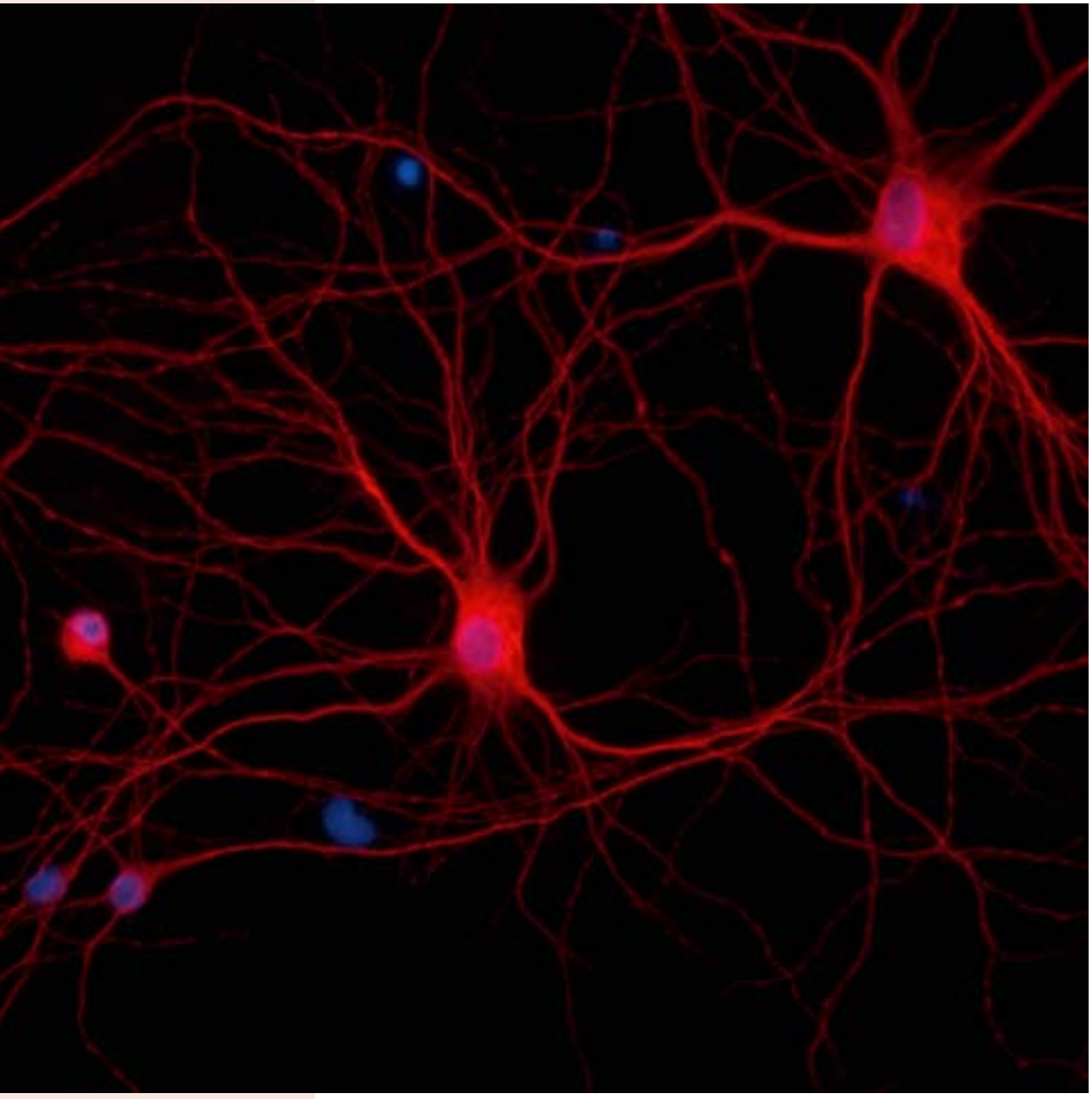


Fig. 4. Benchmarks for the DIBER program. DIBER takes an input mtz file and predicts on the basis of cell constants and structure factors alone whether a crystal contains only protein (green), protein and DNA (red), or only DNA (blue). Classification is done at all costs (a), or with greater than 80% (b) or 90% (c) classification probability.



Culture of thalamic neurons stained for NeuN (author: Andrzej Nagalski)

Laboratory of **Neurodegeneration**

Lab Leader:

Jacek Kuźnicki, PhD, Professor

Associate Professor:

Urszula Wojda, PhD, DSc. Habil.

Post-doctoral Fellows:

Joanna Gruszczyńska -Biegała, PhD

Anna Skibińska-Kijek, PhD (until March 2009)

Adam Sobczak (until Sep. 2009)

Tomasz Węgierski, PhD (since Nov. 2009)

Marta Wiśniewska, PhD

Wojciech Michowski, PhD thesis – Dec. 2009

Junior Researchers:

Emilia Białopiotrowicz, MSc; Katarzyna Dębowska, MSc; Katarzyna Misztal, MSc; Andrzej Nagalski, MSc; Aleksandra Szybińska, MSc; Bożena Żebrowska (until June 2009)

MSc Student:

Mateusz Ambrożkiewicz

Current affiliations of former lab members:

Mateusz Ambrożkiewicz, BSc/Engineer, experiments for diploma were performed at IIMCB, under supervision of Dr. W. Michowski and Prof. J. Kuźnicki. In March 2010 qualified for the MSc/PhD Program in Neurosciences conducted jointly by Georg-August University Göttingen, International Max Planck Research School, the German Primate Center and European Neurosciences Institute in Göttingen

Bożena Kuźniewska, MSc thesis supervised by Dr. hab. U. Wojda. Currently a PhD student at the laboratory of Prof. Leszek Kaczmarek, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology

Łukasz Bojarski, PhD thesis supervised by Prof. J. Kuźnicki. Currently a research group leader, New Therapies of Neurological Diseases, Celon Pharma, www.celonresearch.com

Wojciech Michowski, PhD thesis supervised by Prof. J. Kuźnicki. Currently a postdoctoral research fellow, laboratory of Dr. Piotr Siciński, Department of Cancer Biology, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston MA

Adam Sobczak, PhD thesis supervised by Dr. hab. U. Wojda. Currently a postdoctoral research fellow, Institute of Genetics and Biotechnology, Warsaw University, and Technology Transfer Unit of BioCentrum Ochota.

Jacek Kuźnicki
PhD, Professor

DEGREES

- Professor, 1993
- DSc. Habil., Nencki Institute of Experimental Biology PAN, Warsaw, Poland, 1987
- PhD in biochemistry, Nencki Institute of Experimental Biology PAN, Warsaw, 1980
- MSc in biochemistry, Warsaw University, 1976

POST-DOCTORAL TRAINING

1981-1984 Visiting Fellow, Laboratory of Cell Biology headed by E.D. Korn, National Institutes of Health, Bethesda, MD, 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 located at the Nencki Institute of Experimental Biology PAN, Warsaw

1999-2001 Acting Director, IIMCB; Organizer and Director of Centenarian Program

1996-2002 Head of Laboratory of Calcium Binding Proteins, the Nencki Institute of Experimental Biology PAN, Warsaw

1992-1995 Visiting Professor at the National Institute of Mental Health, Laboratory of Clinical Science, Bethesda, MD, USA

1991-1992 Deputy Director (Scientific Director), Nencki Institute of Experimental Biology PAN, Warsaw

1986-1992 Associate Professor and Head of Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology PAN, Warsaw

1984-1985 Research Associate, Nencki Institute of Experimental Biology PAN, Warsaw

1981-1984 Visiting Fellow, National Institute of Health, Laboratory of Cell Biology, Bethesda, MD, USA

1980-1981 Post-doctoral Fellow, Nencki Institute of Experimental Biology PAN, Warsaw

1976-1980 PhD Student, Nencki Institute of Experimental Biology PAN, Warsaw

MEMBERSHIP IN SCIENTIFIC SOCIETIES, ORGANIZATIONS AND PANELS

- Member of Health Research Advisory Group the 7th FP European Commission, since 2006
- Member of the Polish Academy of Sciences (PAN), since 2004
- Member of the American Society for Biochemistry and Molecular Biology, since 2003
- Head of the Advisory Board of the Centre for Innovative Bioscience Education (SFN), since 2002
- Member of the Biochemical Society (England), since 1995
- Member of the Polish Neuroscience Society, since 1991
- Member of the Polish Society for the Advancement of Science and Arts, since 1991
- Vice-president of the Polish Biotechnology Committee, 1996-1999 and 2000-2002
- Member of the Polish Biotechnology Committee, 1990-2002
- Co-Editor of Advances in Biochemistry (published in Polish), 1989-1992
- Member of the Polish Biochemical Society, since 1977, General Secretary, 1989-1991

HONORS, PRIZES, AWARDS

- Officer's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland, 2008
- Professorial Subsidy Program Award from Foundation for Polish Science (FNP), 2004-2008
- Prime Minister Award for the scientific achievements, 2003
- Award from the Division of Biological Sciences of PAN for the work on calcium binding proteins, 2001
- Knight's Cross of the Order of Polonia Restituta awarded by the President of the Republic of Poland, 1998
- Polish Anatomical Society Award for the article on calcium binding proteins published in "Advances in Cell Biology", 1987
- Skarżyński Award from Polish Biochemical Society for the best review article in Advances in Biochemistry, 1986
- Parnas Award from Polish Biochemical Society for the publishing of the best paper in biochemical research, 1977
- Mozołowski Award, Polish Biochemical Society for outstanding Polish young biochemists, 1977
- MSc, Magna cum laude, University of Warsaw, 1976

Selected publications

- **Wisniewska M**, **Misztal K**, **Michowski W**, **Szczot M**, Purta E, Lesniak W, **Klejman M**, Dąbrowski M, Filipkowski R, **Nagalski A**, Mozrzymas J, **Kuznicki J**. LEF1/beta-catenin complex regulates transcription of the Cav3.1 calcium channel gene (Cacna1g) in thalamic neurons of the adult brain. J Neurosci, 2010; 30:4957-69
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- **Klejman M**, **Gruszczynska-Biegala J**, **Skibinska-Kijek A**, **Wisniewska MB**, **Misztal K**, **Blazejczyk M**, **Bojarski L**, **Kuznicki J**. Expression of STIM1 in brain and puncta-like colocalization of STIM1 and ORAI1 upon depletion of Ca2+ store in neurons. Neurochem Int, 2009; 54:49-55
- **Skibinska-Kijek A**, **Wisniewska MB**, **Gruszczynska--Biegala J**, Methner A, **Kuznicki J**. Immunolocalization of STIM1 in the mouse brain. Acta Neurobiol Exp (Wars), 2009; 69:413-428
- Zekanowski C, **Wojda U**. Aneuploidy, chromosomal missegregation, and cell cycle reentry in Alzheimer's disease. Acta Neurobiol Experiment 2009, 69(2):232-253, review.
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- *Peng H, Lewandrowski U, Müller B, Sickmann A, Walz G, **Wegierski T**. Identification of a Protein Kinase C-dependent phosphorylation site involved in sensitization of TRPV4 channel. Biochem Biophys Res Commun, 2010; 391:1721-5
- *Gao H, Wang Y, **Wegierski T**, Skouloudaki K, Pütz M, Fu X, Engel C, Boehlke C, Peng H, Kuehn EW, Kim E, Kramer--Zucker A, Walz G. PRKCSH/80K-H, the protein mutated in polycystic liver disease, protects polycystin-2/TRPP2 against HERP-mediated degradation. Hum Mol Genet, 2010; 19:16-24
- *Ganner A, Lienkamp S, Schäfer T, Romaker D, **Wegierski T**, Park TJ, Spreitzer S, Simons M, Gloy J, Kim E, Wallingford JB, Walz G. Regulation of ciliary polarity by the APC/C. Proc Natl Acad Sci U S A. 2009; 106:17799-804
- ***Wegierski T**, Steffl D, Kopp C, Tauber R, Buchholz B, Nitschke R, Kuehn EW, Walz G, Köttgen M. TRPP2 channels regulate apoptosis through the Ca2+ concentration in the endoplasmic reticulum. EMBO J. 2009; 28:490-499
- ***Wegierski T**, Lewandrowski U, Müller B, Sickmann A, Walz G. Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. J Biol Chem, 2009; 284: 2923-33.

*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Current Projects

We are interested in molecular mechanisms involved in neurodegeneration and in learning and memory. We study these processes at the genomic, proteomic, and cellular levels. Our major projects include the following:

1. Search for biomarkers and potential therapeutic targets in Alzheimer's disease
   - 1.1. Analysis of the cell cycle in human lymphocytes
   - 1.2. Analysis of mutated p53 in human lymphocytes
   - 1.3. Study on cyclin-dependent kinase 5 in pathogenesis of Alzheimer's disease
2. Calcium homeostasis and calcium signaling in neurons:
   - 2.1. Role of STIM proteins in Store Operated Calcium Entry
   - 2.2. Function of calmyrins in physiology and pathology
3. Role and regulation of β-catenin/Lef1 complex in mature neurons
4. Characterization of biological function of morgana, a CHORD containing protein.

1. Search for biomarkers and potential therapeutic targets in Alzheimer's disease

1.1. Analysis of the cell cycle in human lymphocytes ***(Emilia Białopiotrowicz, Bożena Kuźniewska, under supervision of Urszula Wojda)***

Mounting evidence indicates that the aberrant expression of cell cycle molecules in the brain contributes to the development of Alzheimer's disease and causes neuronal death. Moreover, some molecular changes in Alzheimer's disease can be observed not only in neurons, but also in peripheral cells such as lymphocytes. We have shown that lymphocytes contain proteins characteristic of Alzheimer's disease, such as presenilins (Bojarski et al., Clinical Chem and Lab Med, 2007). Thus, human lymphocytes have potential diagnostic value. Additionally, because of difficulties in studying dynamic processes in postmortem material, such peripheral cells have been used as a model to study molecular mechanisms of Alzheimer's disease. The aim of this study was to determine whether cell cycle alterations can be observed in lymphocytes from patients with sporadic and familial forms of Alzheimer's disease (SAD and FAD). Immortalized with EB-virus, B-lymphocytes from 18 SAD and 8 FAD subjects (with distinct PS1 mutations) were compared with lymphocytes from 33 age-matched healthy individuals. The cell cycle was studied by flow cytometry, real-time PCR arrays, and immunoblotting. Our results revealed discrepancies in the

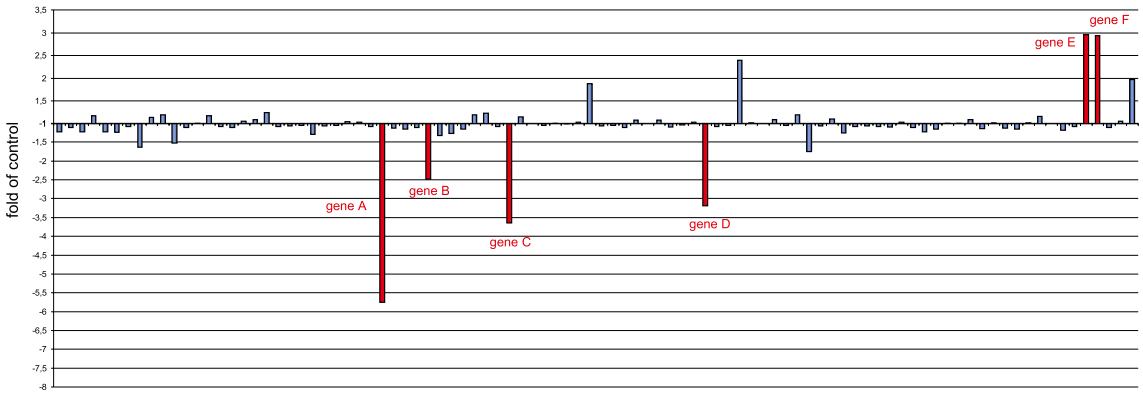


Fig. 1. Expression profiles of 92 cell cycle related genes in immortalized B-lymphocytes from Alzheimer's disease patients (n=3). Analysis was performed twice, using quantitative Real Time PCR arrays (*TaqMan® Express Plate*). The fold change of mRNA copy number compared to control (non-demented individuals, n=3) was calculated by the Ct comparative method. Up-regulated and down-regulated genes in AD lymphocytes are shown, bars represent mean fold of change of each gene in AD versus control after normalization to GAPDH expression. Data for genes selected as potential AD markers are shown in red (author: Bożena Kuźniewska).

cell cycle regulatory transcriptome and proteome between SAD and FAD cells, indicating that the mechanism of SAD differs from FAD (manuscript submitted).

Furthermore, we assessed the influence of nine differentially located PS1 mutations (P117R, M139V, L153V, H163R, S170F, F177L, I213F, L226F, and E318G) on the cell cycle in human non-neuronal cells. The experiments were carried out in the immortalized lymphocytes and in transiently and stably transfected HEK-293 cells with wildtype and PS1 mutants. We also introduced PS1 mutants in HEK-293 cells stably co-expressing APP with Swedish mutation (APPsw) to measure the level of $A\beta_{1-40}$ and $AA\beta_{1-42}$ peptides. Our data demonstrated that PS1 mutations had various impacts on the cell cycle that strongly depended on the mutation location in the PS1 molecule. We analyzed correlations between cell cycle changes and the clinical parameters of FAD patients and the levels of secreted $A\beta_{1-40}$ and $A\beta_{1-42}$ (manuscript in preparation). Altogether, these data indicate that cell cycle proteins in human lymphocytes from Alzheimer's disease patients can be further assessed as possible diagnostic markers (manuscript in preparation).

1.2. Analysis of mutated p53 in human lymphocytes *(Aleksandra Szybińska in cooperation with Maurizio Memo and Daniela Uberti, University of Brescia)*

Our collaborative studies revealed an increased level of conformationally altered p53 protein in immortalized B-lymphocytes from patients with sporadic and familial Alzheimer's disease compared with lymphocytes from healthy controls. The conformational p53 mutant may be useful as a marker to discriminate Alzheimer's disease from non-Alzheimer's disease patients (Lanni et al., Mol Psychiatry, 2008). The p53 conformational tertiary structure is influenced by the redox status of the cells, and evaluation of the oxidative profile of these patients was performed. We found that among the markers of oxidative stress, hydroxytransnonenal modified proteins were significantly increased in FAD patients. Interestingly, in addition to increased levels of oxidative markers, the antioxidant defense mechanisms were compromised in these patients due to decreased levels of enzymes such as superoxide dismutase. These results support and enhance the first evidence of peripheral unfolded p53 associated with Alzheimer's disease pathology and indicate that immortalized peripheral cells from Alzheimer's disease patients are good models for identification of disease markers.

1.3. Study on cyclin-dependent kinase 5 in pathogenesis of Alzheimer's disease *(Aleksandra Szybińska in collaboration with Aleksandra Wysłouch-Cieszyńska and Michał Dadlez from the Mass Spectrometry laboratory, Institute of Biochemistry and Biophysics PAN)*

Cyclin-dependent protein kinase 5, in complex with its brain-specific activator p35, was recently shown to be involved in a variety neuronal processes in both developing and adult brains. In Alzheimer's disease patients, the brain expression and activation of cdk5 was also shown to be upregulated. The consequences of that upregulation, other than coparticipation in MAP tau overphosphorylation, are poorly understood. We used a proteomics method to analyze protein expression and modifications in synaptosomes of transgenic mice (i.e., Alzheimer's disease models bearing human mutated presenilin 1 and APP genes). Samples from animals at three different ages (3, 6, and 14 months) were analyzed. Using iTRAQ reagent peptide labeling followed by separation of the labeled peptide mixture in a pH gradient by isoelectric focusing, we were able to identify over 3000 synaptic proteins and a set of differential proteins that were up- or downregulated in synaptosomes in transgenic animals. The oldest mice showed the highest degree of protein expression dysregulation. Additionally, to reveal differences in protein phosphorylation between transgenic and wildtype animals, metal ions and metal oxide affinity chromatography methods are being optimized to purify phosphorylated peptides from digested synaptosomal proteins.

2. Calcium homeostasis and calcium signaling in neurons

2.1. Role of STIM proteins in Store Operated Calcium Entry *(Joanna Gruszczyńska-Biegała, Aleksandra Szybińska, Tomasz Węgierski)*

We are studying the mechanisms of calcium homeostasis in neurons and its dysregulation in neurodegenerative diseases. In non-excitable cells, cooperation of ER calcium sensors STIM1 or STIM2 with the plasma membrane calcium channel protein ORAI1 was discovered to be crucial for the proper functioning of Store Operated Calcium Entry (SOCE), also known as capacitative calcium entry (CCE). However, little is known about these proteins in excitable cells. We tested the hypothesis that the mechanism of SOCE in neuronal cells is based on STIM proteins and that alterations of SOCE may lead to pathologies such as Alzheimer's disease.

We identified STIM1 and STIM2 proteins in mouse brain and in cultured cortical and hippocampal neurons using various techniques. Analysis of the puncta in thapsigargin (TG)-treated cells revealed that store depletion increases puncta-like colocalization of STIM1 and ORAI1 more than STIM2 and ORAI1. In an attempt to understand the differential localization of STIM1 and STIM2 in the brain and the mechanism of their translocation in neurons, we further studied the effects of simultaneous expression of ORAI1 and STIM1 or ORAI1 and STIM2 on SOCE in cortical neurons. We measured intracellular calcium levels during SOCE using a Ca2+ imaging method and FURA-2. This analysis was performed after depletion of intracellular Ca2+ stores by treating cells with TG in Ca2+-free medium and during subsequent incubation of neurons in 2 mM Ca2+ media. Our results demonstrate that SOCE is enhanced in neurons transfected with STIM1 and ORAI1, but not with STIM2 and ORAI1. We show that in neurons, similar to non-excitable cells, the ORAI1 and STIM proteins are involved in SOCE and that STIM1 and STIM2 have different functions (Skibinska-Kijek et al., Acta Neurobiol Exp (Wars), 2010).

To analyze alterations of SOCE (CCE) in Alzheimer's disease, we used EBV-immortalized lymphocytes from Alzheimer's disease patients. We studied the interaction of STIM 1 and ORAI 1 in lymphocytes of Alzheimer's disease patients compared with healthy controls. We used the in situ Proximity Ligation Method, which enables detection and visualization of interactions between the two proteins. In sites of protein interaction, single fluorescent points appear, thus allowing interaction quantification.

2.2. Function of calmyrins in physiology and pathology *(Katarzyna Dębowska, Magdalena Blażejczyk, Adam Sobczak, under supervision of Urszula Wojda)*

Ca^{2+} ions play a role as a second messenger in multiple neuronal signaling pathways to regulate development, plasticity, and cell death and are also involved in the pathogenesis of neurodegenerative diseases. The EF-hand proteins, such as calmodulin (CaM) and neuronal calcium sensors (NCS), are the major effector proteins translating the chemical signal of increased Ca^{2+} concentration into diverse biochemical responses in neurons. Our research concentrates on a novel family of EF-hand Ca^{2+}-binding proteins called calmyrins (CaMy, known also as KIP or CIB). Specifically,

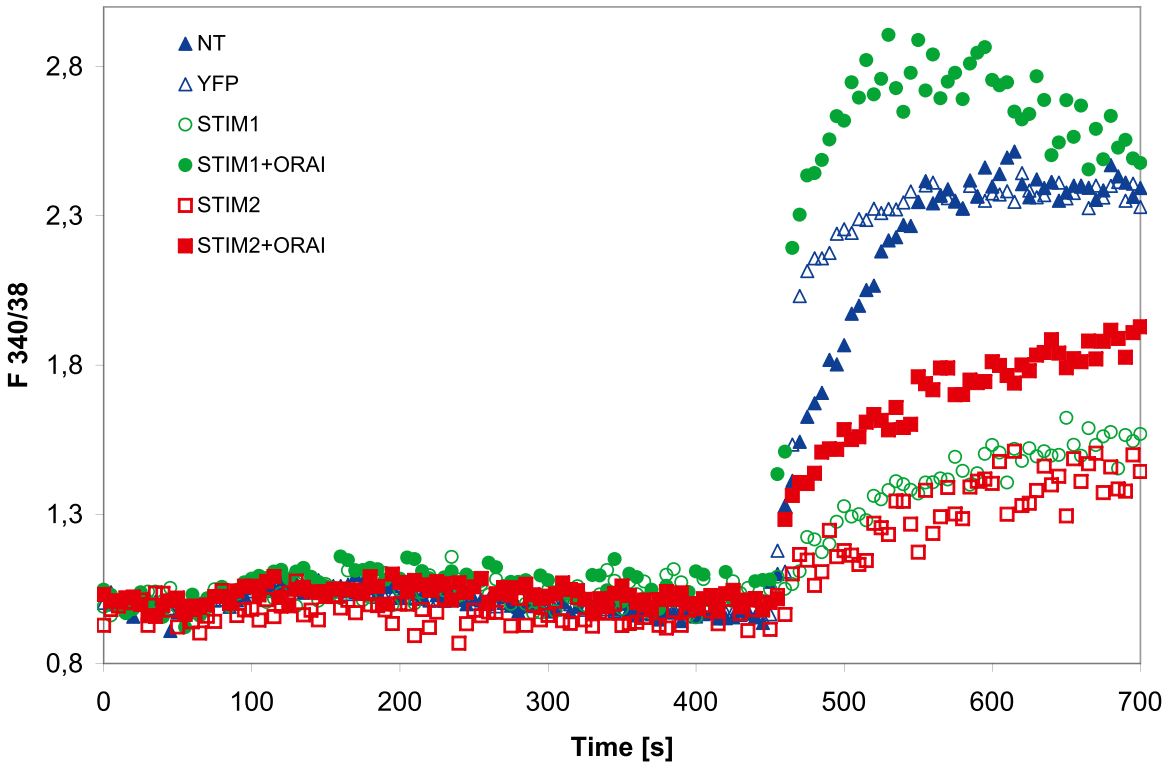


Fig. 2. Analysis of CCE in transfected cortical neurons in response to store depletion. Averaged traces obtained by ratiometric Fura-2AM analysis of neurons overexpressing YFP-STIM1 ± ORAI1, YFP-STIM2 ± ORAI1, YFP, or not transfected. Measurements began in a buffer supplemented with 0.5 mM EGTA, which was replaced 1 min later by buffer with 0.5 mM EGTA supplemented with 1 μM Tg. After an additional 6.5 min, the medium was replaced by buffer with 2 mM CaCl2 (author: Joanna Gruszczyńska-Biegała in colaboration with Paweł Bieganowski from the Nencki Institute).

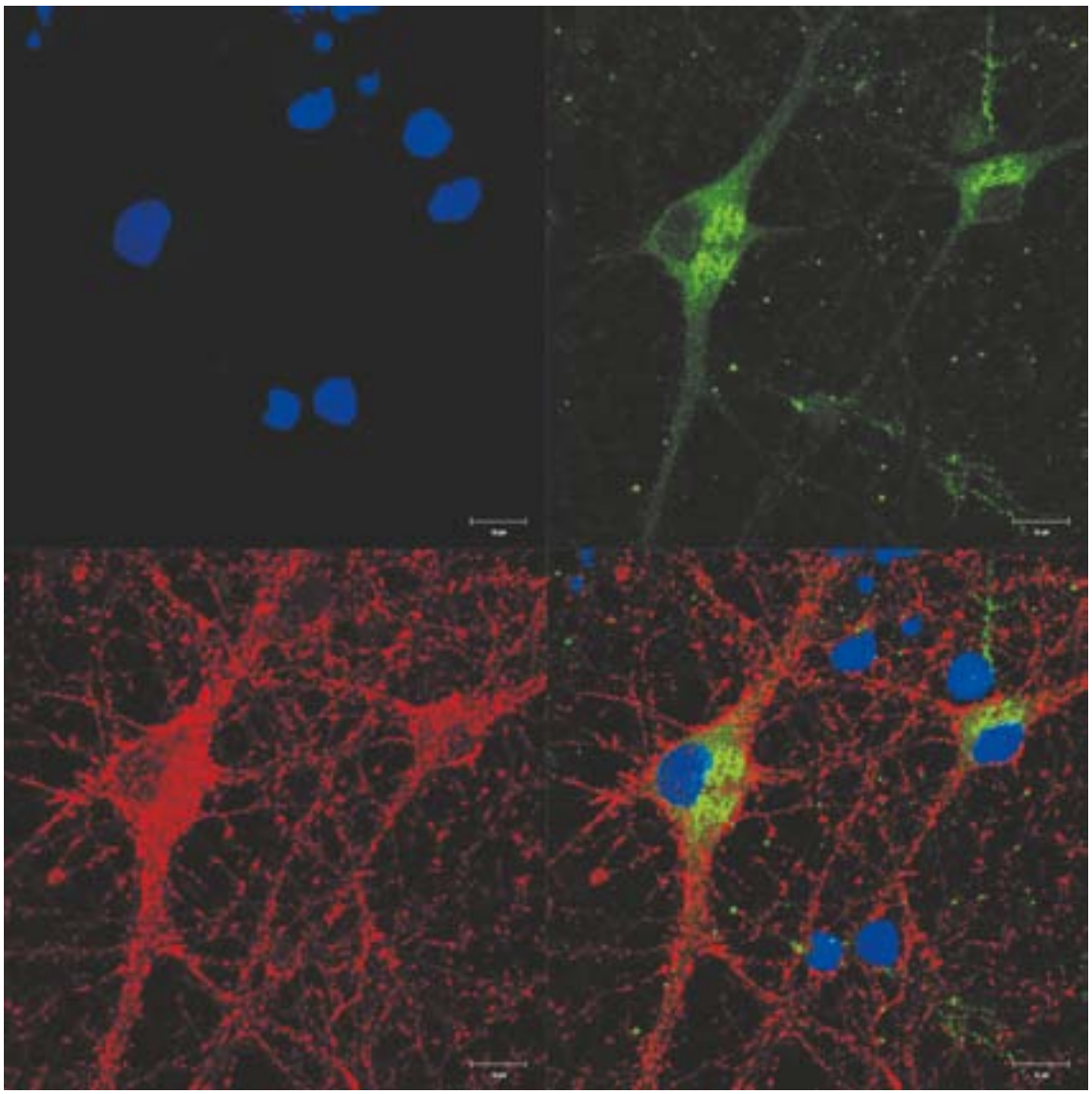


Fig. 3. Immunolocalization of Calmyrin2 (*CaMy2*) and its potential binding partner in 17DIV hippocampal neurons stimulated with NMDA. Alexa 488 (CaMy2), Alexa 568 (*potential binding partner*), Hoechst (*nuclei*). Scale bar 10 µm (author: Katarzyna Dębowska).

we seek to elucidate the functions of CaMy1 and CaMy2 in neurons by analyzing CaMy1 and CaMy2 localization, biochemical properties, and protein ligands in the brain. In collaboration with the group of Michael Kreutz from the Leibnitz Institute, Magdeburg, we previously demonstrated that CaMy1 is implicated in Alzheimer's disease (Blazejczyk and Wojda, Calcium Binding Proteins, 2008; Blazejczyk et al., Biochim Biophys Acta, 2006; Bernstein et al., Neuropathol Appl Neurobiol, 2005; Sobczak et al., Acta Biochim Pol, 2005). We have searched for possible protein ligands of CaMy1 using a two-hybrid yeast screen. We identified a new potential target of CaMy1 in neurons and showed that this CaMy1 interaction is calcium-dependent. We confirmed this interaction in neurons by coimmunoprecipitation, coimmunolocalization, and the Proximity Ligation Method. We are currently investigating the functional role of this new CaMy1 interaction using PC12 cells and cultured primary hippocampal neurons (Sobczak et al., in preparation).

Moreover, we pursued studies on rat CaMy2. We cloned CaMy2 from rat brain and demonstrated that CaMy2 binds Ca^{2+} and exhibits a Ca^{2+}/conformational switch. Moreover, CaMy2 undergoes N-myristoylation without a Ca^{2+}/myristoyl switch, is membrane-associated, and localizes in neurons together with the Golgi apparatus and dendrite markers. The CaMy2 transcript and protein were detected mainly in the hippocampus and cortex of rat brain. Studies on rat primary hippocampal neurons conducted in collaboration with Dr. Jacek Jaworski from the Laboratory of Molecular and Cellular Neurobiology showed that CaMy2 expression is induced upon neuronal activation. The CaMy2 induction was blocked by translation inhibitors, specific NMDAR antagonists, the Ca^{2+}-chelator BAPTA, and inhibitors of ERK1/2 and PKC, kinases transmitting NMDAR-linked Ca^{2+} signaling. Our results show that CaMy2 levels are controlled by NMDAR and Ca^{2+} and suggest a role for CaMy2 in Ca^{2+} signaling underlying NMDAR activation (Blazejczyk et al., Arch Biochem Biophys, 2009). We also identified new potential targets of CaMy2 in rat brain by affinity chromatography followed by mass spectrometry and confirmed these interactions using several in vitro methods. The physiological significance of these interactions in primary neurons is currently under investigation (Debowska et al., in preparation).

3. Role and regulation of β-catenin/Lef1 complex in mature neurons *(Katarzyna Misztal, Andrzej Nagalski, Marta Wiśniewska)*

β-catenin is a protein that functions in two cellular compartments. Membranous β-catenin is involved in cell-cell adhesion via cadherins, whereas in the nucleus, it activates LEF1/TCF transcription factors as a mediator of the Wnt signaling pathway. In the developing and adult brain, nuclear β-catenin activates genes involved in the proliferation and differentiation of neuronal precursor cells.

Unknown is whether β-catenin plays a role in transcription regulation in mature neurons. This issue is important because Wnt signaling has been implicated in some neurodegenerative and mental disorders. Recently, we found that in the adult mouse brain, β-catenin and LEF1 accumulate in the nuclei of neurons specifically in the thalamus and regulate the expression of the Cacna1g gene encoding the Cav3.1 T-type channel, which is involved in epilepsy. In collaboration with Jerzy Mozrzymas and Marcin Szczot from Wroclaw Medical University, we showed that activation of Wnt/β-catenin signaling leads to T-type current increases in thalamic cells (Wisniewska et al., J Neurosci, 2010).

Presently, we have two main lines of research on β-catenin in the adult brain. In the first project, we are looking for the β-catenin target genes in neurons. We use PCR arrays to profile gene expression in the brain and in adenovirus-transduced primary neurons. In silico analyses are performed in collaboration with Michal Dabrowski from Nencki Institute, Warsaw. To experimentally confirm the actual targets, we perform luciferase assays, footprinting, and chromatin immunoprecipitation. In the second project, we are exploring the mechanisms of cytoplasmic/nuclear β-catenin stabilization in specific brain regions. We are looking for β-catenin-interacting proteins using pull-down and mass-spectrometry techniques.

4. Characterization of biological function of morgana, a CHORD-containing protein in the nervous system *(Wojciech Michowski, Marta Wiśniewska, Mateusz Ambrożkiewicz in collaboration with Guido Tarone and Mara Brancaccio from the University of Turin and Barbara Zabłocka from Mossakowski Medical Research Center)*

CHORD (Cys and His Rich Domain) contains a novel type of zinc finger. In plants, these domains are involved in defense against pathogens. The human genome has two genes encoding CHORD-containing proteins, melusin and morgana/CHP-1. Melusin is present exclusively in cardiac and skeletal muscles. It protects the heart from the consequences of chronic aortic hypertension. Morgana/CHP-1 has been recently reported to control centrosome duplication and to be necessary for proper cell division (Ferretti et al., Dev Cell, 2010, in press). The highest level of morgana/CHP-1 is found in the brain, which is a largely postmitotic tissue. Thus, the biological role of this protein is likely to reach beyond the regulation of cell division. The Morgana/CHP-1 gene is regulated by HSF-1 (Heat Shock Factor 1) in response to temperature stress, and the protein interacts directly with heat shock protein 90 (HSP90; Hahn, FEBS Lett, 2005), suggesting the involvement of morgana/CHP-1 in the cell response to stress. We showed that morgana/CHP-1 prevents a thermoliable protein from aggregation in vitro, indicating its molecular chaperone activity. Moreover, fibroblasts overproducing morgana/CHP-1 were less sensitive to different stressors. Importantly, we showed that the morgana/Chp-1 spatial expression pattern in response to stress overlaps with naturally resistant areas in vivo. Morgana/Chp-1 expression was induced in the gerbil hippocampus after transient global brain ischemia, and this upregulation was long-lasting in the naturally ischemia-resistant region of the hippocampal formation (Michowski et al., submitted).

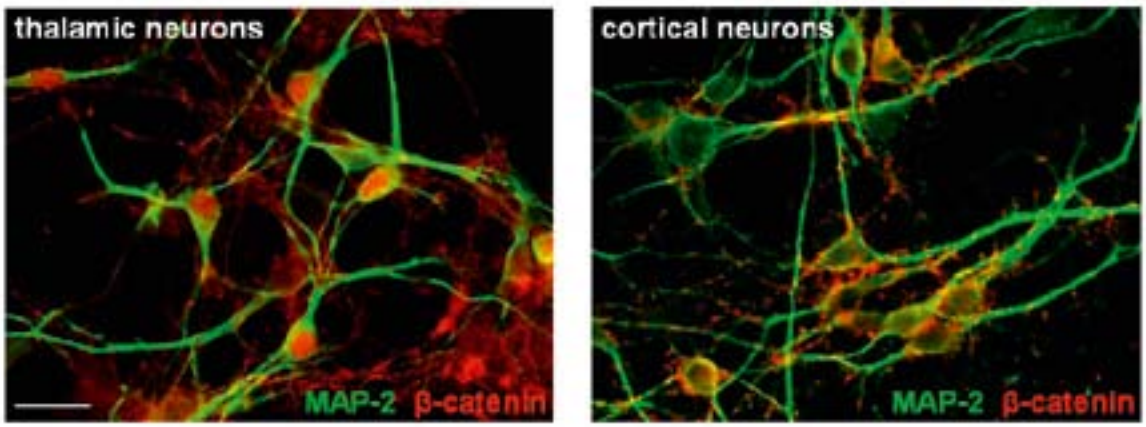
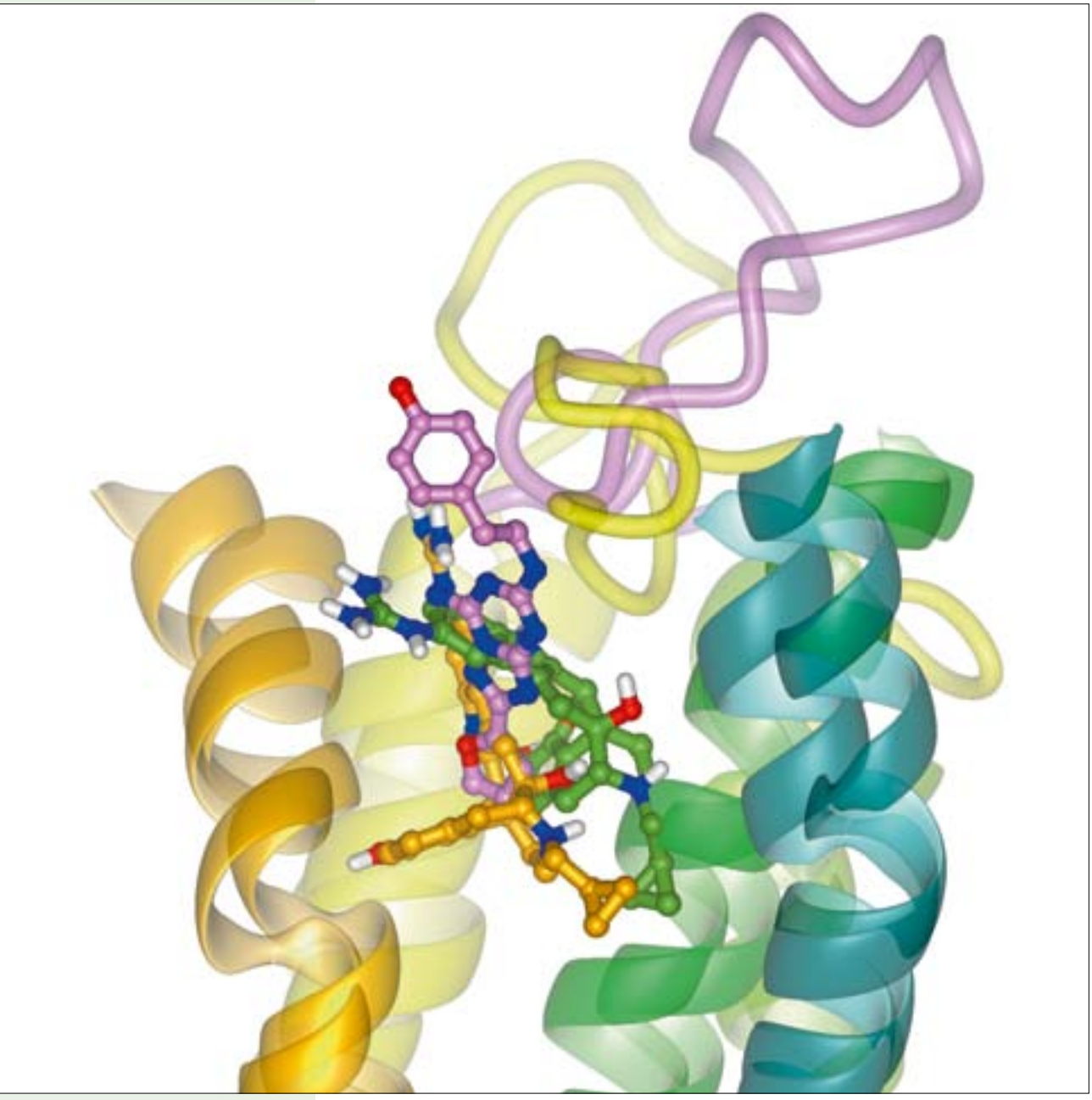
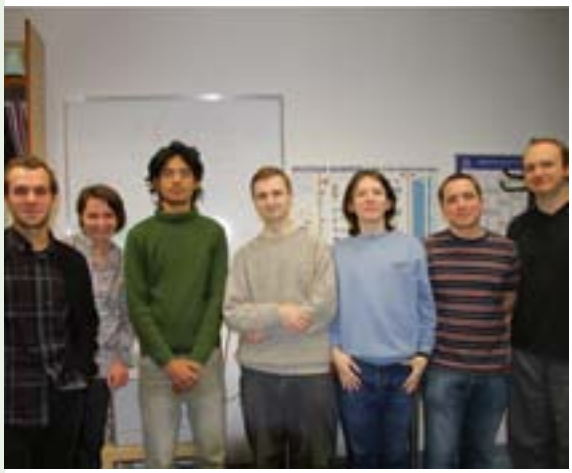


Fig. 4. Spontaneous accumulation of β-catenin in nuclei of thalamic neurons.
Thalamic and cortical primary cultures were labeled with β-catenin-specific mouse monoclonal antibody (red staining) and neuronal marker MAP2-specific rabbit polyclonal antibody (green staining) (author: Katarzyna Misztal).



Superimposition of κOR agonist 6′-GNTI (carbon atoms in orange) and κOR antagonist 5′-GNTI (carbon atoms in green) models and the crystal structure of $A_{2a}R$ with inverse agonist (carbon atoms in violet). For clarity, part of the helices and all loops, with the exception of EL2, were removed. The position and conformation of EL2 (shown in yellow for κOR, violet for $A_{2a}R$) allow diffusional motion of ligands to and from both receptors (author: Sławomir Filipek).

Laboratory of **Biomodelling**



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DEGREES

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HONORS, PRIZES, AWARDS

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PROFESSIONAL MEMBERSHIPS

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- Biophysical Society
- Polish Society of Medicinal Chemistry
- Polish Bioinformatics Society

EDITORIAL BOARD MEMBER

- Journal of Bionanoscience
- The Open Structural Biology Journal

PUBLICATIONS

- over 70 publications in primary scientific journals
- over 1900 citations
- 7 publications cited more than 100 times

Selected publications

- **Jozwiak K**, **Krzysko KA**, Bojarski L, Gacia M, **Filipek S**. Molecular models of the interface between anterior pharynx-defective protein 1 (APH-1) and presenilin involving GxxxG motifs. ChemMedChem, 2008; 3:627-634
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- **Kolinski M**, **Filipek S**. Structurally similar pair of agonist and antagonist of kappa opioid receptor studied by molecular dynamics simulations. J Mol Model, 2010; accepted.
- **Kolinski M**, **Filipek S**. Studies of the Activation Steps Concurrent to Ligand Binding in DOR and KOR Opioid Receptors Based on Molecular Dynamics Simulations. The Open Struct Biol J (TOSBJ), 2009; 3:51-63
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Current Research

1. Studies of activation mechanisms in opioid receptors

Opioid receptors belong to family A (rhodopsin-like) of G-protein-coupled receptors (GPCRs). There are four types of opioid receptors: δOR (OP_1), κOR (OP_2), μOR (OP_3), and the nociceptin/opioid receptor-like 1 (OP_4). They are involved in pain modulation and in a number of physiological functions and behavioral effects. Understanding opioid receptor sensing, activation, and signaling is greatly important especially because such mechanisms may be more general and operate also in other GPCRs. The results may have significant implications for the discovery and development of more specific medicines to treat GPCR-linked dysfunctions and diseases involving blindness, diabetes, allergy, depression, cardiovascular defects, and some forms of cancer. Although GPCRs interact with very diverse sets of ligands, the membranous part of GPCRs shares extensive similarities, having seven transmembrane helices linked by relatively short loops. Each receptor undergoes a series of conformational rearrangements controlled by molecular switches leading to partial or full activation. The dynamic character of GPCRs is hypothesized to be essential for their diverse physiological functions.

Among the structurally similar guanidinonaltrindole (GNTI) compounds, 5'-GNTI is an antagonist and 6'-GNTI is an agonist of κOR (Fig. 1). To explore how a subtle alteration of the ligand structure influences receptor destiny, we investigated two concurrent processes: the final steps of ligand binding in the receptor binding site and the initial steps of receptor activation. To trace such early activation steps, the membranous part of the receptor was built on an inactive receptor template, whereas the extracellular loops were built using the ab initio CABS method. We used the simulated annealing procedure for ligand docking and all-atom molecular dynamics simulations to find the immediate changes in the structure of the ligand-receptor complex. Binding of an agonist, contrary to an antagonist, induced a break in the "3-7 lock" between helices TM3 and TM7 (Fig. 2). We also observed an action of the extended rotamer toggle switch which can suggest interdependence between these two switches.

The observed binding modes for structurally similar agonist-antagonist pairs and early activation steps confirm our earlier findings obtained for μOR, δOR, and κOR. The current results were collected for two different models of κOR with docking performed independently so positions of the same ligands were different, although the distinctive features of agonist vs. antagonist binding were preserved. The antagonist was bound with its tyramine group to Y3.33(139) on helix TM3, whereas the antagonist to H6.52(291)

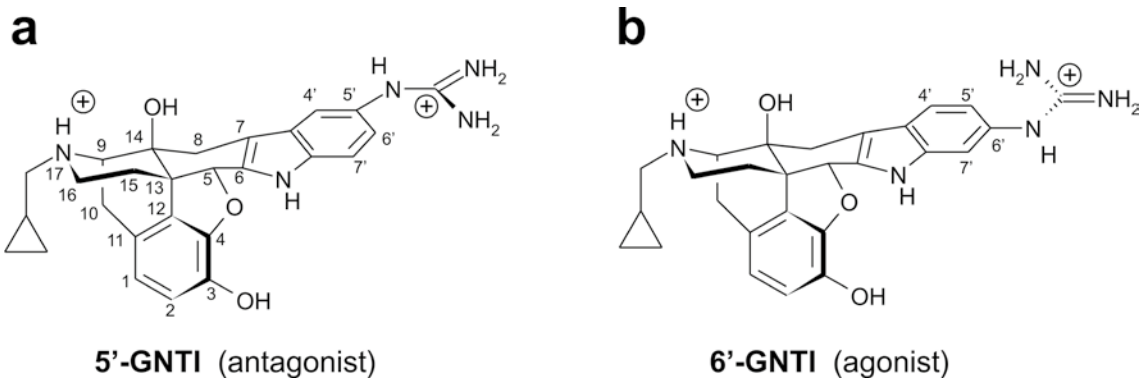


Fig. 1. Structural formulas of antagonist 5'-GNTI (a) and agonist 6'-GNTI (b) of the κOR. Numbering of atoms is shown for the first compound (author: Sławomir Filipek).

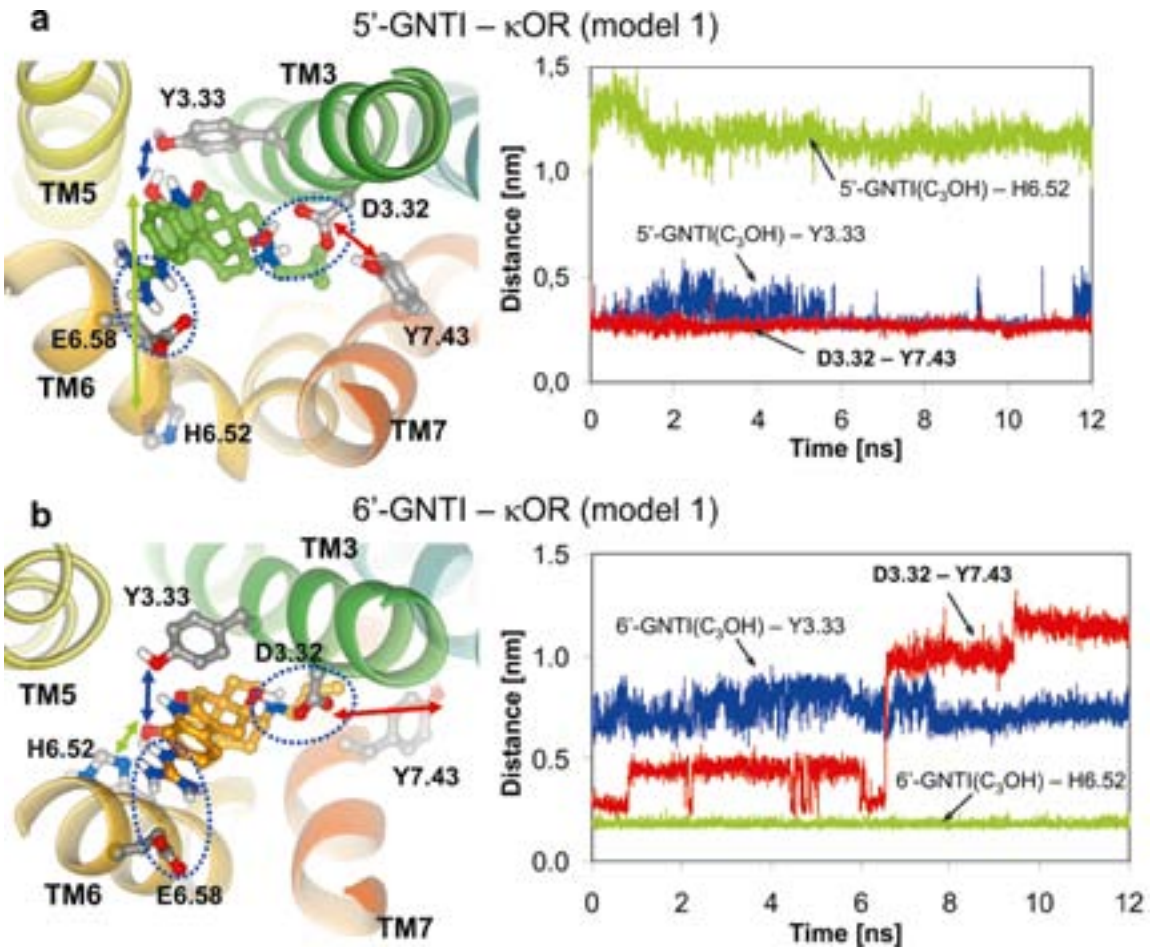


Fig. 2. Binding modes of ligands in the κOR binding site. (a) Antagonist 5'-GNTI. (b) agonist 6'-GNTI. Blue dashed ellipses denote ligand-receptor ionic interactions. View from extracellular side. The right side shows plots of selected distances within the binding site between ligand and receptor: D3.32[138]-Y7.43[320] "3-7 lock" in red, ligand(C3OH)-Y3.33[139] in blue, and ligand(C_3OH)-H6.52[291] in green. (author: Sławomir Filipek).

was bound on helix TM6. In all cases, the guanidinium group was bound to E6.58(297) on helix TM6. Activation events, breaking of the "3-7 lock" (between helices TM3 and TM7), and an extended rotamer toggle switch action were evoked only in the case of the 6'-GNTI agonist, despite the similarity of shapes and electrostatic properties between 6'-GNTI and 5'-GNTI. To differentiate between such tight ligand pairs, the sensing mechanism in the binding site of the receptor must operate on an extremely confined area such as that one we proposed. Our studies were conducted on monomeric receptor structures in agreement with the recent findings for μOR that the monomer is a minimal unit to bind ligands and activate G proteins. However, dimerization may additionally modify receptor-ligand interactions and activation routes, rendering cross-talk between GPCRs exceptionally complicated. Elucidation of the mechanisms of ligand binding and early activation steps may lead to a better understanding of the onset of signaling processes and to more efficient drug design.

2. Chemokine receptors

Chemokines belong to a large family of small, chemotactic cytokines that regulate the trafficking of immune cells by binding to cell surface receptors belonging to the GPCR superfamily. Chemokines coordinate the homeostatic circulation of leukocytes and their movement to sites of inflammation or injury. There are approximately 50 chemokines and 20 different receptors identified to date. The largest family of chemokines is characterized by two cysteines adjacent to each other in the sequence (the CC family). There is a total of 28 different CC chemokine ligands (CCL1-CCL28) and 10 corresponding CC chemokine receptors (CCR1-CCR10). Dysregulated expression of chemokines and their receptors is involved in the development of many human diseases, including immunodeficiency, autoimmune, and chronic inflammatory diseases and cancer. As a consequence, considerable efforts have been made to develop drugs that modulate the activity of chemokines and their receptors. The goal of this project is to develop and use computational strategies and techniques to ab initio predict the tertiary (three-dimensional) structure of CC family chemokine receptors (Fig. 3).

3. Stability studies of rhodopsin—prototype of GPCRs

Many GPCRs are palmitylated at cysteine residues in the carboxyl terminal region. Unclear is the precise role of palmitylation at both the structural and functional levels. This fatty acid linkage has been implicated in various facets of GPCR function, including coupling to and activation of the heterotrimeric G protein, receptor phosphorylation, internalization, and desensitization. Some receptors are dynamically palmitylated, whereas others are constitutively

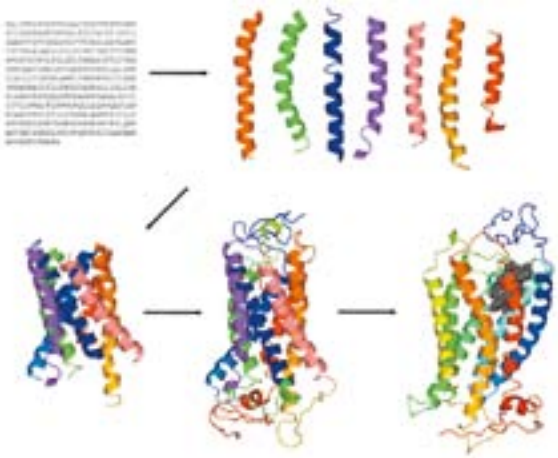


Fig. 3. Schematic illustration of the *ab initio* method of predicting chemokine receptors and their receptor-ligand complexes. The schematic begins with the amino acid sequence (a), followed by building single helices (b), combining helices into bundle (c), building interhelical loops between transmembrane helices (d), and predicting the ligand-binding site and ligand-receptor affinity (e) (author: Bartosz Trzaskowski).

palmitylated. No consensus has been reached among GPCRs concerning the role that palmitylation plays in receptor structure and function, which may reflect the different roles this covalent modification plays in different systems. Rhodopsin is palmitylated at two cysteine residues in its carboxyl terminal region. The structure of rhodopsin is largely unperturbed by the absence of palmitate linkage. The largest effect observed at both the structural and functional levels occurred near the carboxyl terminal region of the receptor where the covalent fatty acid linkage occurs. Single-molecule force spectroscopy revealed that the largest effect on the rhodopsin structure occurs at the carboxyl terminal region in the absence of palmitylation. This region became less stable in unpalmitylated rhodopsin. The absence of palmitylation in rhodopsin, therefore, hinders the activation of transducin by light-activated rhodopsin, which may be a result of the destabilization that occurs in the carboxyl terminal region.

Simulations were conducted to test the influence of palmitylation on the structure of rhodopsin. Thirty-two independent simulations were conducted for 30 ns each: 16 simulations of rhodopsin with palmitylation and 16 simulations of rhodopsin without palmitylation. To determine the effect of palmitylation on the structure of rhodopsin surrounding the region of the palmitate groups, several angles were chosen and analyzed. Molecular dynamics calculations confirmed that the unpalmitylated rhodopsin structure was only slightly changed compared with wildtype (Fig. 4), especially in the amphiphilic helix 8 region. Although the absence of palmitylation in rhodopsin alone may not critically affect human vision under normal conditions, it may have more drastic consequences under prolonged extreme lighting conditions or under conditions in which alterations or mutations in other molecules or proteins involved in phototransduction or the retinoid cycle occur.

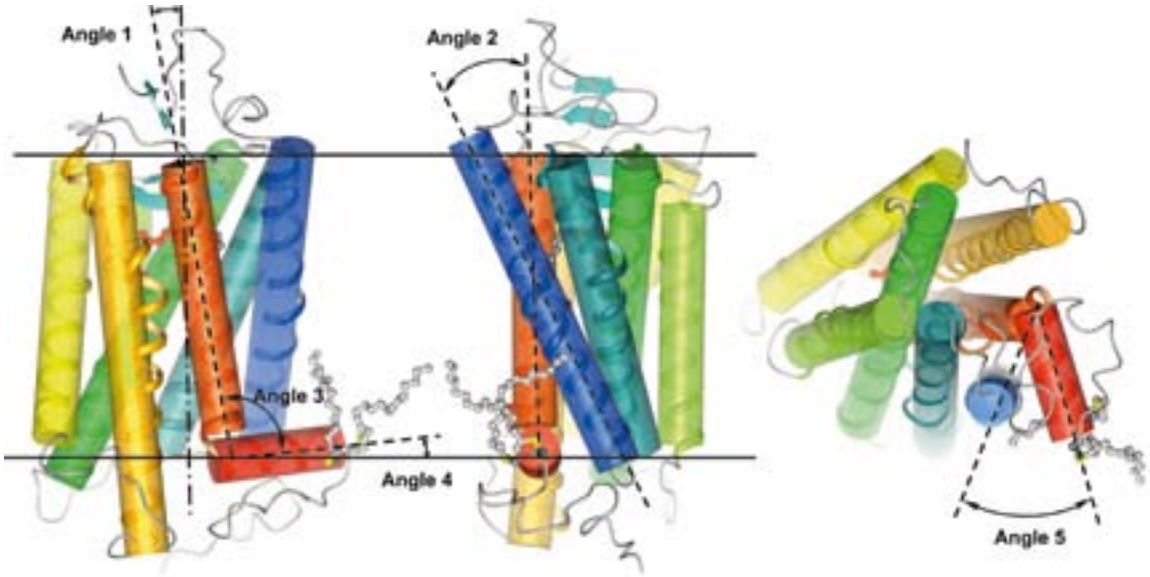
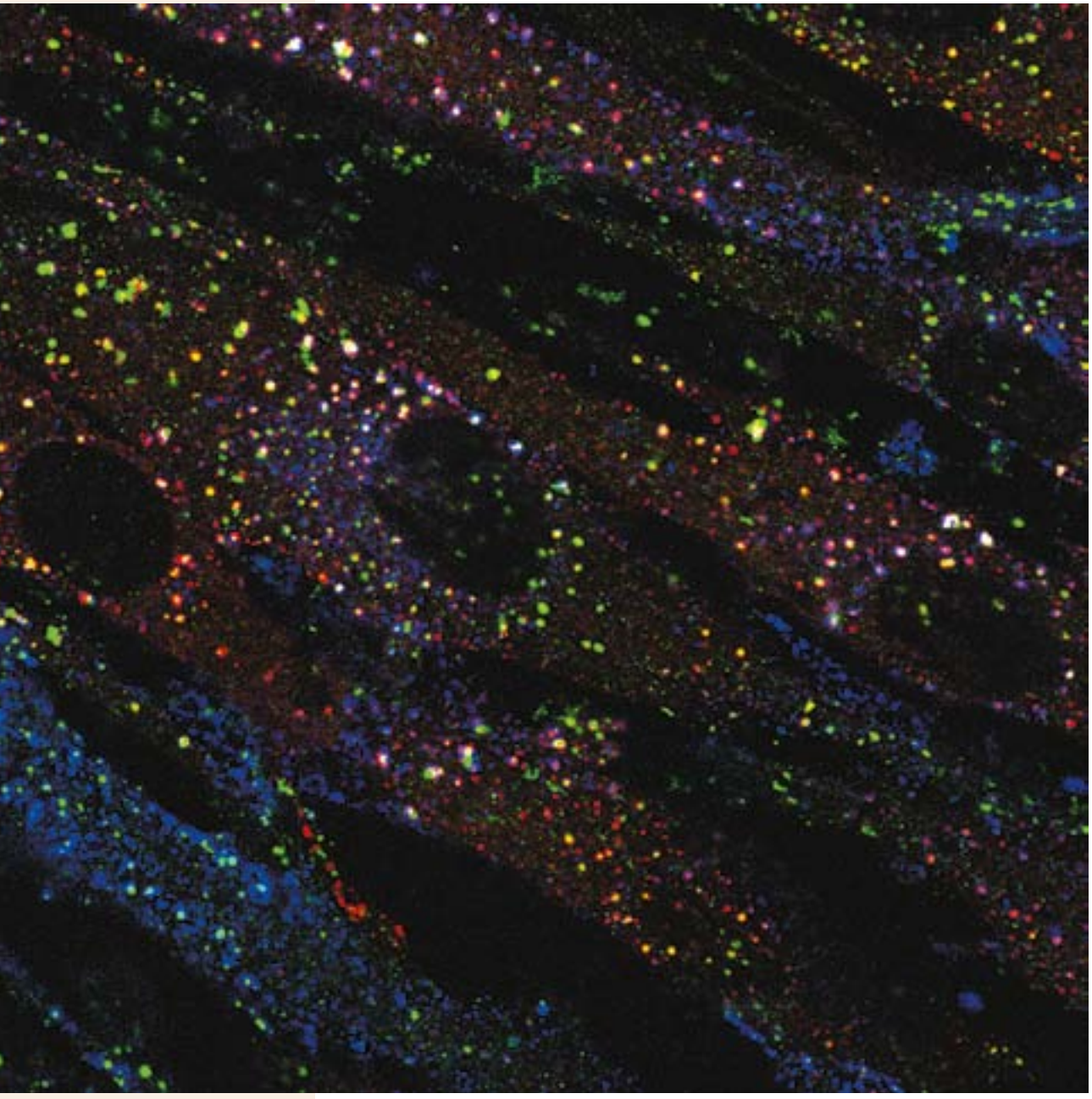


Fig. 4. Effect of palmitylation assessed by molecular dynamics simulations. The analyzed angles are highlighted in the figure (angles 1-5). The change of rhodopsin structure after removal of palmitates was visible only for angles 3-5: angle 3 (helix 7 to helix 8), 90.3° → 97.7°, angle 4 (helix 8 to membrane plane), 8.2° → -0.2° (minus means that helix 8 is directed out of the membrane), and angle 5 (cytoplasmic end of helix 1 to helix 8), 51.5° → 55.5°. Helices are depicted in a color spectrum from blue (helix 1) to orange-red (helix 7) and red (helix 8) (author: Sławomir Filipek).



Human skin fibroblasts with internalized platelet-derived growth factor (PDGF, green) and transferrin (blue) localized in endosomes. The receptor for PDGF is stained in red (author: Lukasz Sadowski)

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1993	MSc in molecular biology, Jagiellonian University, Cracow, Poland
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RESEARCH TRAINING

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1997-2000	postdoctoral training at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany
1993-1996	PhD studies in the Institute of Microbiology and Genetics, University of Vienna, Austria
1990-1991	Exchange Student at the University of Wolverhampton, Wolverhampton, UK

FELLOWSHIPS AND AWARDS

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

Selected publications

- **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
- **Olchowik M**, **Miaczynska M**. Effectors of GTPase Rab5 in endocytosis and signal transduction. Postepy Biochem, 2009; 55:171-180
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- **Pilecka I**, **Banach-Orlowska M**, **Miaczynska M**. Nuclear functions of endocytic proteins. Eur J Cell Biol, 2007; 86:533–547
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*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Description of Current Research

The Laboratory of Cell Biology focuses on studying the relationship between the processes of intracellular signal transduction and membrane trafficking. Our aim is to elucidate the molecular mechanisms by which endocytic transport regulates signal transmission and affects final signaling output. We use cultured mammalian cells as our basic experimental model, to which we apply a variety of biochemical, microscopic, and functional assays. The specific projects developed by our group follow two general lines of investigation, with the aim of clarifying the following:

1. **The role of endosomal compartments in trafficking and signaling of growth factors.**
2. **The involvement of endocytic proteins in the regulation of gene expression in the nucleus.**

At a general level, both topics investigate molecular communication between intracellular organelles in endocytic membrane transport and signal transduction. This is a novel issue of increasing significance in the field of cell biology. Many recent studies, including our own, indicate that 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 downregulation of surface receptors and their degradation. 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 (Miaczynska et al., Curr Opin Cell Biol 2004). 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; Fig. 1).

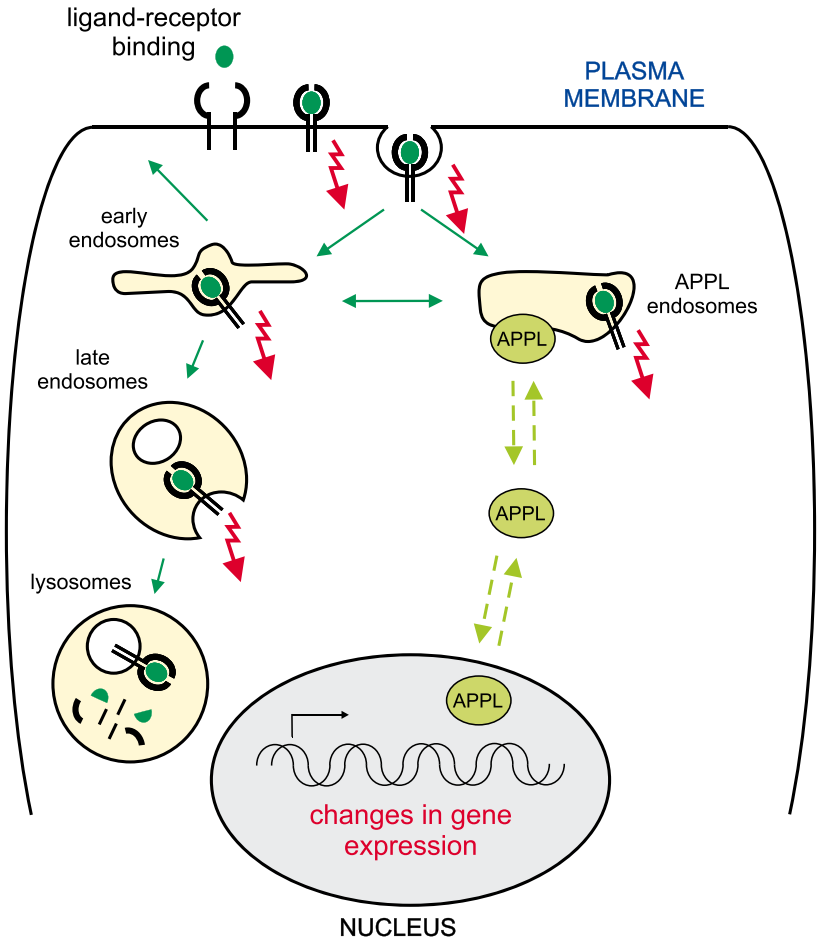


Fig. 1. Schematic of cargo trafficking and signaling along the endocytic pathway (author: Marta Miaczynska). According to the classical view, ligand-receptor complexes are internalized from the plasma membrane via clathrin-coated vesicles (CCV) to early endosomes from where they are sorted either toward recycling endosomes back to the plasma membrane (not shown) or to late endosomes and lysosomes for degradation. Our work indicates that APPL-harboring compartments represent a distinct subpopulation of early endosomes, receive cargo from the plasma membrane via CCV, and exchange it with the canonical early endosomes. In addition to its endosomal localization, APPL proteins can undergo nucleocytoplasmic shuttling and interact with nuclear proteins, thus modulating gene expression. Signal transduction, initiated by signaling ligands binding to their receptors at the plasma membrane, can continue intracellularly from endosomal compartments during trafficking (signaling events marked with red arrows).

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 of 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 research lines were the previous studies of adaptor proteins APPL1 and APPL2. These homologous endosomal proteins also act as signal transducers capable of nuclear translocation, thus providing examples of both the involvement of endosomes in signaling and the activity of endocytic proteins in the nucleus (Miaczynska et al., Cell 2004; Fig. 1). APPL1 and APPL2 are effectors of the small GTPase Rab5, a key regulator in the early steps of endocytosis. They are localized to a subpopulation of Rab5-positive endosomes that appear segregated from the well-characterized canonical early endosomes marked by another Rab5 effector, EEA1. Interestingly, APPL proteins can be released from the endosomal membrane, undergo nucleocytoplasmic shuttling, and interact with nuclear proteins, among them the histone deacetylase and chromatin remodeling complex NuRD. Knockdown of APPL1/APPL2 proteins by RNAi demonstrated that each is required for efficient cell proliferation. By identifying the endocytosis regulator Rab5 and the nuclear chromatin remodeling complex NuRD as interacting partners of both APPL proteins, these data suggested a novel molecular link between the processes of endocytosis and chromatin remodeling. Moreover, APPL-harboring endosomes appeared as an intermediary step in signaling between the plasma membrane and the nucleus. 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 toward exploring other dual-function endocytic proteins. Below are two examples of our recently completed projects, which document the roles of APPL proteins in transcriptional regulation and nuclear signaling.

APPL1 and APPL2 are novel activators of β-catenin/TCF-mediated transcription

In the search for proteins interacting with APPL1 and APPL2, we identified Reptin, a multifunctional ATPase of the AAA+ family. Reptin and a closely related protein, Pontin, act together in a dodecameric complex in several processes related to chromatin remodeling and transcriptional control, but they also exhibit separate or even antagonistic functions. We showed that both APPL proteins interacted directly with Reptin, and this interaction was mapped to the pleckstrin homology (PH) domain of APPL1. Confocal microscopy experiments demonstrated that although Reptin did not colocalize with APPL1 on endosomal structures, both proteins were present in the cell nucleus and cytoplasm, indicating that their interaction may occur in these compartments. However, no interaction between APPL1 and Pontin could be detected, suggesting that APPL proteins may regulate a specific function of Reptin that does not involve Pontin. One such function is an association between Reptin and β-catenin, histone deacetylases (HDAC1 and HDAC2), and Groucho/TLE, which leads to transcriptional repression of β-catenin targets in the canonical Wnt signaling pathway. Consistent with this hypothesis, endogenous APPL1 was found in a complex containing Reptin, HDAC1/2, and β-catenin.

To test the possible involvement of APPL proteins in the transcriptional regulation of β-catenin targets, reporter gene assays were performed employing a TOPFLASH plasmid containing the luciferase gene under the control of TCF-responsive elements. Overexpression of APPL1 or APPL2 stimulated the activity of the TCF-dependent reporter construct, whereas silencing of APPL1 reduced it. Consistent with previously published reports, overexpression of Reptin repressed TCF-dependent transcription via associated HDAC enzymes. Co-overexpression of Reptin with either APPL1 or APPL2 attenuated Reptin-mediated repression in a manner depending on the dose of APPL1/2, suggesting that APPL protein binding reduces the repressive effect of Reptin. To clarify the molecular mechanisms underlying this effect, the composition of the complex comprising Reptin, HDACs, and β-catenin under conditions of APPL1 or APPL2 overexpression was analyzed. Interestingly, overexpression of either APPL protein reduced the amounts of HDAC1 and HDAC2 associated with Reptin and β-catenin. Furthermore, the lower amounts of HDACs in Reptin immunoprecipitates resulted in reduced HDAC activity present in the complex measured by a fluorimetric assay of deacetylase enzymatic activity. Finally, increased levels of APPL proteins stimulated the expression of Wnt target genes (e.g., c-jun, c-myc, or CCND1 [cyclin D1]) and lowered the amounts of Reptin and HDAC1 present on the CCND1 or Axin2 gene promoters detected by chromatin immunoprecipitation.

Overall, these results identified APPL proteins as novel components that play a role in Wnt/β-catenin signaling by regulating TCF-dependent transcription. In particular, the mechanism by which APPL proteins exert their stimulatory effects on β-catenin/TCF-dependent transcription involves decreasing the repressive activity of a Reptin- and HDAC-containing complex (Rashid et al., J Biol Chem 2009; Fig. 2).

In general, these data indicate a possible link between Rab5-dependent endocytic processes and canonical Wnt signaling and provide an interesting example of the involvement of endocytic proteins in transcriptional control. We are currently extending our studies to other proteins implicated in endocytosis and capable of nucleocytoplasmic

shuttling by performing systematic screens for the involvement of candidate proteins in transcriptional regulation mediated by several transcription factors.

Functional characterization of the interactions between endosomal adaptor protein APPL1 and the NuRD co-repressor complex

APPL1 was previously shown to interact with the NuRD (nucleosome remodeling and deacetylase) complex (Miaczynska et al., Cell 2004), but the biochemical basis or functional relevance of this interaction remained unknown. This multiprotein co-repressor complex is unique with respect to combining the two usually separate activities of chromatin remodeling and histone deacetylation in one macromolecular assembly. These activities are provided by the nucleosome remodeling ATPase Mi-2 and two related class I HDACs (histone deacetylases), HDAC1 and HDAC2, respectively. The main function of the NuRD complex is transcriptional repression mediated by HDAC1 and HDAC2, which are involved in deacetylation of histone H3 and H4 tails. Overall, NuRD regulates fundamental cellular processes, such as proliferation and differentiation, and thus plays an important role in development and carcinogenesis.

We further characterized binding between APPL1 and NuRD. Depletion of HDAC2 by RNAi precluded an interaction between APPL1 and the NuRD complex, thus indicating that HDAC2 is a key NuRD subunit responsible for the association with APPL1. Interestingly, HDAC1 could not compensate for the lack of HDAC2 in supporting the interaction between APPL1 and NuRD, which strongly argues for their non-redundant roles within the NuRD complex. HDAC2-dependent interactions between APPL1 and NuRD were detected both in cytoplasmic and nuclear fractions and in the presence of HDAC inhibitors, demonstrating that the enzymatic activity of HDAC2 is not a prerequisite for binding to APPL1. Nevertheless, under normal conditions and in the absence of any inhibitors, APPL1 interacted with the NuRD complex containing enzymatically active HDAC2, but not HDAC1, as the only deacetylase.

One unresolved issue regarding the endocytic proteins acting in the cell nucleus is the mechanism underlying their nuclear translocation. Some of these endocytic adaptors possess classical nuclear localization signals (NLS), whereas others enter the nucleus via interactions with NLS-harboring partners. APPL1 lacks a canonical NLS, and its nuclear translocation must occur via a piggy-back mechanism through binding with NLS-harboring partners. We discovered that interactions with the NuRD complex promoted the nuclear localization of APPL and that the extent of APPL1-NuRD interactions could be regulated by the cellular levels of HDAC1. Silencing HDAC1 resulted in increased binding of APPL1 to NuRD, which in turn promoted the nuclear localization of APPL1. HDAC1 overexpression decreased binding of APPL1 to NuRD and led to lower levels of APPL1 present in the cell nucleus. Finally, although we were unable to detect any measurable changes in the properties of the HDAC2-containing NuRD complex upon alterations of APPL1 levels in cells, we nevertheless observed the impact of APPL1 on the interactions exhibited by HDAC1. Overexpression of APPL1 reduced the binding of HDAC1 with the other core NuRD subunits and correlated with the increased in vivo expression of $p21^{WAF1/CIP1}$, a gene specifically repressed by HDAC1 under normal conditions. Silencing of APPL1 exerted an opposite effect and resulted in reduced expression of $p21^{WAF1/CIP1}$. These data suggest that increased APPL1 levels negatively modulate the repressor potential of HDAC1, indicating that APPL1 may act as a sequestering factor for HDAC1 to restrict its function in vivo. Altogether, these data reveal the surprising complexity of APPL1 interactions with histone deacetylases, with functional consequences for the modulation of gene expression (Banach-Orlowska et al., Biochem J 2009). More broadly, these results contribute to an emerging theme of endocytic proteins playing alternative roles in the cell nucleus.

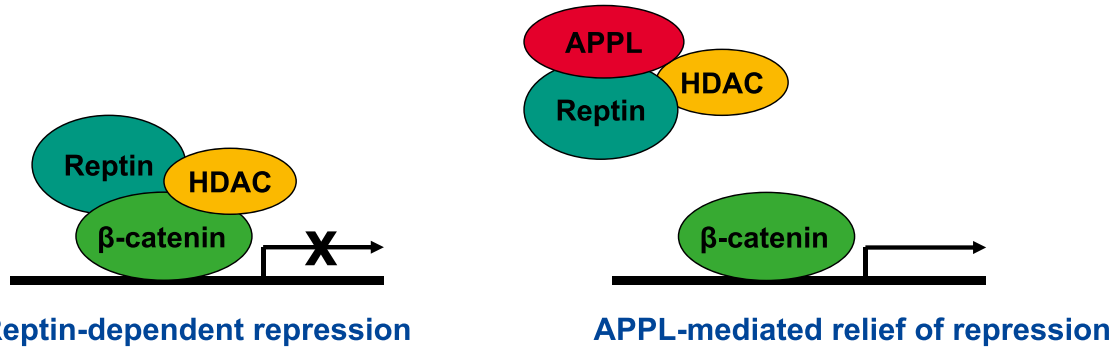
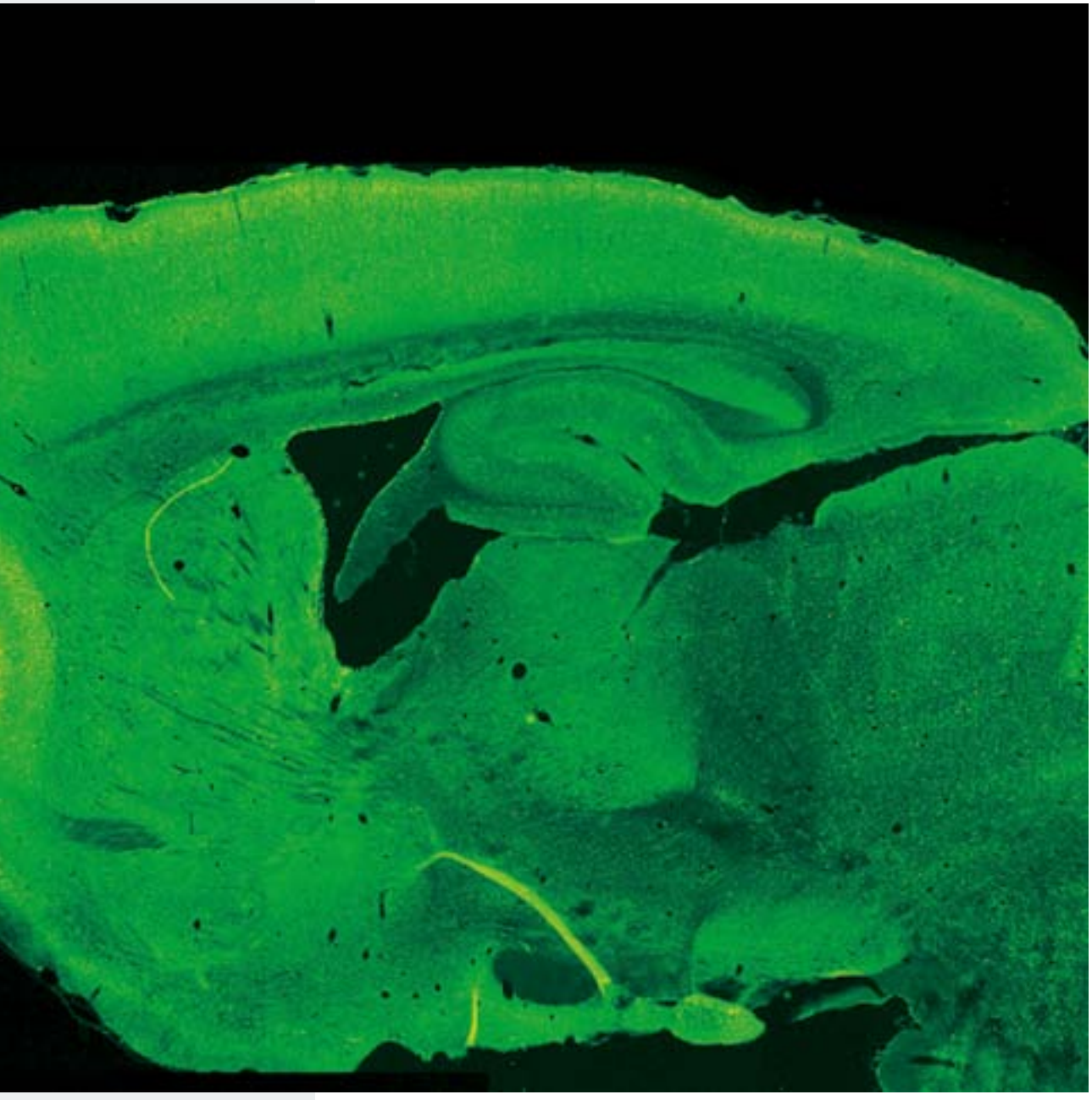


Fig. 2. Schematic depicting the mechanism by which APPL proteins can mediate attenuation of Reptin- and HDAC-mediated transcriptional repression of Wnt/β-catenin target genes (author: Marta Miaczynska).



Seizure-induced gene expression activation in the brain of transgenic rat carrying cFos-Psd95-Venus transgene (author: Matylda Macias).

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RESEARCH TRAINING

2006	Erasmus Medical Center, Dr. C.C. Hoogenraad, Rotterdam, Holland, research visit, one month
2002-2005	Picower Center for Learning and Memory, Massachusetts Institute of Technology and Howard Hughes Medical Institute, Prof. Morgan Sheng, Cambridge, MA, USA; postdoctoral associate
2000	ARL Division of Neural Systems, Memory and Aging, University of Arizona, Dr. J. Guzowski, Tucson, USA (one month), research training
1997-2001	Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs (LGN.), Prof. J. Mallet, UMR 9923 CNRS, Paris, France (seven months total), research training
1996-2002	Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology PAN, Prof. L. Kaczmarek, Warsaw, Poland; PhD student until 2001; postdoctoral associate until May 2002
1995-1996	Department of Genetics, Prof. P. Węgleński, Warsaw University, Poland, master degree

DEGREES

2001	PhD in molecular neurobiology, Nencki Institute of Experimental Biology PAN, Warsaw, Poland
1996	MSc in Biology, Department of Genetics, Warsaw University, Poland

FELLOWSHIPS AND AWARDS

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

Publications in 2009

- **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
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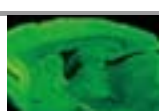
*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Description of Current Research

The research of our team concentrates on mTOR-dependent control of proper neuronal morphology in health and disease. Establishing the proper neuronal morphology is an absolute base for proper brain function. Therefore, mechanisms of axon targeting, dendritic arbor patterning, proper cell contact formation, and maintenance of plasticity of neuronal connectivity are at the center of interest of molecular neurobiology. Dendrites are the main site of information input onto neurons, and different neurons have distinctive and characteristic dendrite branching patterns. Advances in electrophysiology and computational modeling have clearly shown that dendritic arbor shape is one of the crucial factors determining how signals originating from individual synapses are integrated. In fact, several neurodevelopmental pathologies are characterized by abnormalities in the dendritic tree structure. Dendritic arbor development is a multi-step process that is controlled by both external signals and intrinsic genetic programs. Among the proteins that transduce extracellular or cell surface signals into changes in dendritic arbor and dendritic spine shape are several protein kinases. Among them are phosphoinositide 3 kinase (PI3K) and its downstream kinases Akt and mTOR. mTOR is a serine/threonine protein kinase known to merge extracellular instructions with information about cellular metabolic resources and to control the rate of anabolic and catabolic processes accordingly. In neurons, mTOR has been implicated in neuronal differentiation, axon elongation and directional movements, long-term synaptic plasticity, and learning and memory. mTOR is hypothesized to act primarily by phosphorylating eIF-4E binding protein (4EBP) and p70 ribosomal S6 protein kinase (p70S6K), which are important regulators of protein translation. In the context of mTOR involvement in local protein synthesis in neuronal dendrites, our data describing mTOR-4EB-P1 and p70S6 kinase involvement in dendritic branching raise an interesting issue, namely whether local or general mTOR signaling is required for dendrite morphogenesis. This issue serves as a starting point for studying the more general question of the potential role of local protein synthesis in dendritic tree development.

However, "chemical genomics," performed on yeast and in microarray studies with *Drosophila* cells, identified hundreds of Rapamycin-dependent mutants, the analysis of which suggests that mTOR might be involved in cellular functions other than translation, such as transcription, membrane turnover, mitochondrial function, autophagy, and microtubule stability. Almost all of these cellular processes can be potentially important for dendritic branching and spine morphogenesis and stability. Additionally, in mammalian cells, mTOR forms two heteromeric and functionally distinct protein complexes called mTORC1 and mTORC2. mTORC1 is Rapamycin-sensitive and consists of mTOR bound to Raptor. This complex is involved in the control of a wide variety of cellular processes (discussed above). Rapamycin-insensitive mTORC2, containing mTOR and Rictor, regulates actin cytoskeleton dynamics and the activity of protein kinases such



as Akt, PKC, and SGK1. Indeed, our recent findings strongly suggest that both mTORC1 and mTORC2 are needed for proper dendritic branching. Considering the key role that mTOR plays in neuronal physiology, not surprising is that mTOR signaling is disturbed under various neuropathological conditions. Altered mTOR activity has been reported in brain tumors, tuberous sclerosis, cortical dysplasia, and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. However, in cases of either physiological processes or neuropathology, our knowledge of molecular events downstream of mTOR is rather limited. Determining the mTOR-dependent proteins and cellular processes involved in dendritogenesis and synapse formation processes will be important, including which of these are specifically disturbed in brain pathologies. Addressing these issues will be crucial for understanding the molecular biology of neurons and assessing the benefits and risks of expanding the clinical use of mTOR inhibitors. Elucidating these points is a major aim of our team. For the past few years, including 2009, our research has developed in three 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.
3. Characterizing both mTOR-regulated cellular processes and the role of local protein synthesis in pathologies of the central nervous system.

Progress in areas 1 and 3 is discussed in more detail in the following sections.

Identification of mTOR partners and regulated proteins involved in the process of dendritic branching

Our major effort toward identifying mTOR-regulated proteins involved in dendritic arborization has been to design a shRNA library against mRNA encoding those proteins and perform a screen in neurons cultured in vitro. We selected 150 proteins potentially regulated by the mTOR-Raptor complex based on a bioinformatic approach and designed a library of siRNAs against all selected candidates. We next prepared a shRNA-pSUPER plasmid-based library, which consisted of 450 plasmids encoding individual shRNAs against selected genes (three hairpins per sequence). In 2008, we performed a screen and identified 30 genes crucial for dendritic arbor development and stability using this library. In 2009, we validated our positive hits and investigated developmental expression patterns of selected genes. We primarily focused on β-adaptin, mDia and tistetraproline (Fig. 1). Our choice has been motivated by the relatively poor understanding of the

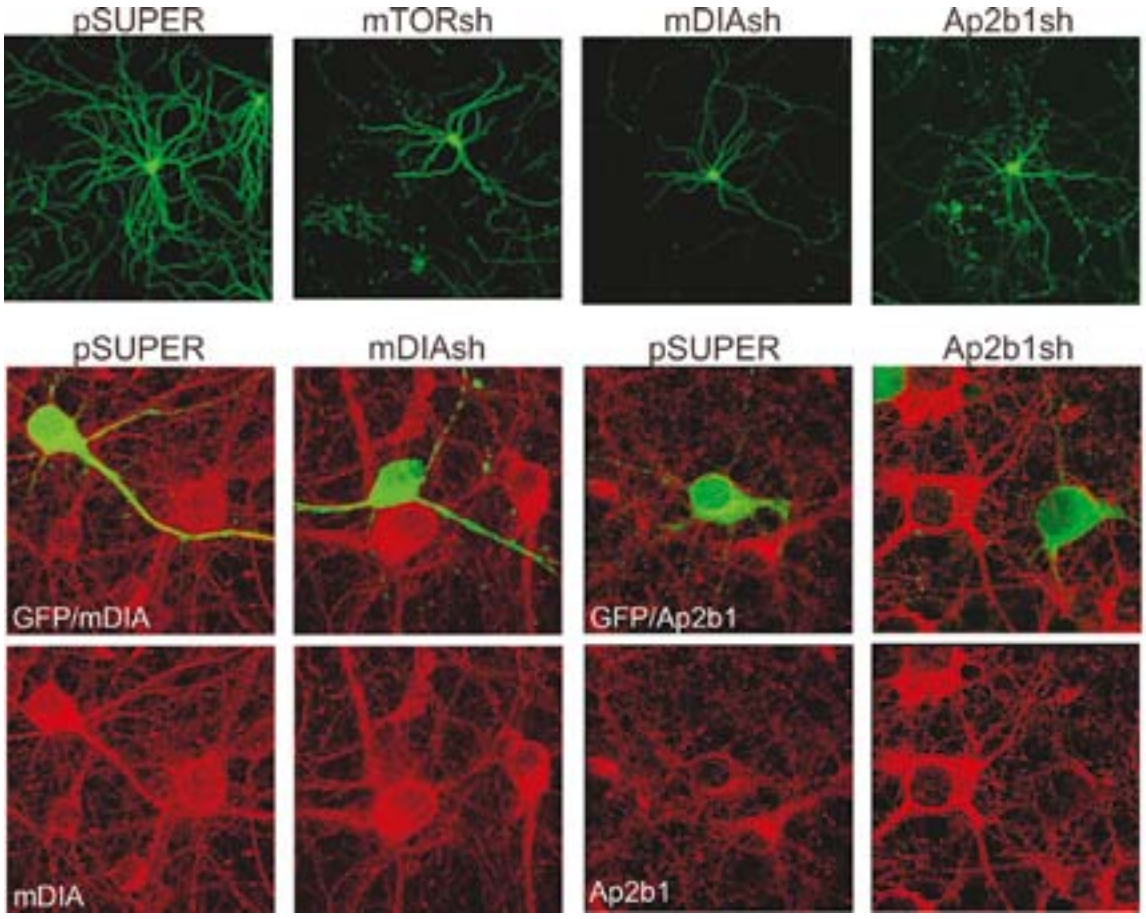


Fig. 1. mDia and β-adaptin proteins indentified as mTOR-dependent regulators of dendritic arborization by a shRNA library screen. Transfection of developing neurons cultured in vitro with pSUPER plasmid encoding shRNAs targeting mDia or b-adaptin for 4 days resulted in a decrease in the number of dendrites, similar to mTOR knockdown (upper panel) and a decrease in endogenous levels of targeted proteins (lower panel) (authors: Anna Malik & Małgorzata Urbańska).

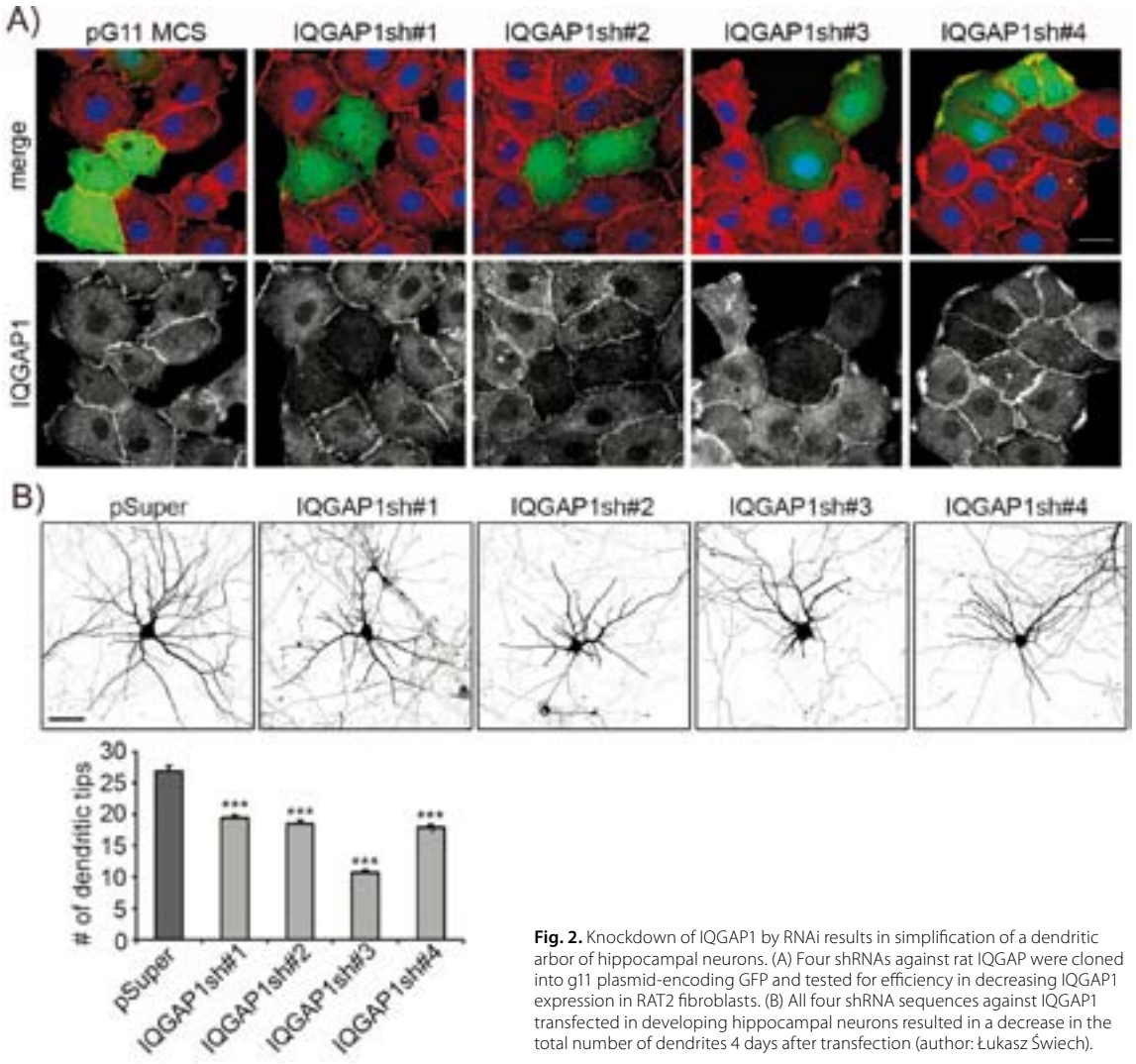


Fig. 2. Knockdown of IQGAP1 by RNAi results in simplification of a dendritic arbor of hippocampal neurons. (A) Four shRNAs against rat IQGAP were cloned into g11 plasmid-encoding GFP and tested for efficiency in decreasing IQGAP1 expression in RAT2 fibroblasts. (B) All four shRNA sequences against IQGAP1 transfected in developing hippocampal neurons resulted in a decrease in the total number of dendrites 4 days after transfection (author: Łukasz Świech).

contribution of cellular processes involving those proteins (i.e., membrane trafficking, actin nucleation, and RNA degradation) to dendritic arbor morphology. An especially interesting case is tistetraproline, a protein involved in mRNA degradation, a process that has yet to be studied in the context of dendritic arbor morphology control. As a next step, we will study in more detail the molecular mechanisms underlying the role of selected genes in dendritogenesis and determine their potential role in neuropathology connected with dendritic arbor disturbances. For example, our microarray analysis of transcriptome after excessive activation of neurons in epilepsy revealed induction of tistetraproline expression.

In 2009, we also significantly advanced our studies on mTOR-dependent regulation of dendritic arborization involving CLIP-170. CLIP170 belongs to a group of microtubule plus-end tracking proteins (+TIPs) and is believed to regulate microtubule dynamics at their plus-ends by promoting their growth. However, CLIP-170 plays additional roles (e.g., bridging microtubules to a cortical actin). Our previous research showed that mTOR and CLIP170 can interact in brain extracts and that inhibition of mTOR activity prevents full phosphorylation of CLIP-170. Moreover, introduction of shRNA against CLIP-170 into rat hippocampal neurons in dissociated and organotypic primary cultures resulted in a significant reduction in the number of dendrites, a decrease in the complexity of dendritic arbors, and shrinkage of dendritic fields. Moreover, CLIP-170 knockdown exerts a strong effect on the shape of dendritic arbors even under conditions promoting dendritogenesis, such as overexpression of constitutively active forms of PI3K and Akt kinases, which are crucial upstream components of the mTOR signaling pathway. In 2009, we studied precisely how CLIP-170 regulates dendritic morphology. We first proved that forced actin polymerization is able to reverse the deleterious effects of CLIP-170 RNAi. Indeed, we found that CLIP-170 interacts with the actin cytoskeleton regulator IQGAP1 in neurons. Curiously, this interaction depended on mTOR activity. Using RNAi technology, we showed that IQGAP1, similar to CLIP-170, is important for proper dendritic arbor morphology (Fig. 2). Moreover, similar to CLIP-170 knockdown, knockdown of IQGAP1 blocked PI3K-induced, mTOR-dependent dendritogenesis (Fig. 2). Altogether, our data strongly suggest that mTOR controls dendritogenesis by facilitating interactions of microtubules with an actin cytoskeleton. Notably, such mTOR function has not yet been described and is potentially interesting also to non-neurobiologists.

Characterization of both mTOR-regulated cellular processes and local protein synthesis: role in pathologies of the central nervous system

Our important aim is to understand how physiological processes regulated by mTOR are disturbed in pathology of the nervous system. In 2009, we continued our search for proteins involved in CNS precursor differentiation and neuronal morphology defects in tuberous sclerosis. However, we also initiated new research related to (i) the molecular role of mTOR in epileptogenesis and (ii) mTOR and GSK3 reciprocal communication in physiology and neurodegenerative disorders.

Epilepsy is a chronic neurological disorder defined by recurrent spontaneous seizures, which are often precipitated by synchronous activity of localized groups of neurons (so called epileptic foci). Temporal lobe epilepsy (TLE) is the most common type of epilepsy in adults. One issue that must be solved in developing effective treatment is how seizure activity progressively alters the temporal lobe to produce an epileptic state. The first new project, which is part of a Polish-Norwegian Research Fund grant, is based on a specific hypothesis for TLE pathogenesis in which seizures stimulate an aberrant form of synaptic plasticity, dynamically engaging four interactive domains of the synapse: presynaptic and postsynaptic compartments, the extracellular matrix (ECM) within the synaptic cleft, and, notably, the thin astrocytic processes ensheathing the synapse. At the molecular level, the key step involves hypertranslation of local dendritic mRNA encoding a network of functionally interconnected molecules. mTOR is implicated as a potential mediator of hyperactivated dendritic protein synthesis in epilepsy. Overactivation of mTOR is reported in brain pathologies associated with seizures (e.g., tuberous sclerosis, PMSE syndrome, cortical dysplasia), whereas pharmacological inhibition of mTOR suppresses seizures in animal models (e.g., TSC knockout mice, kainic acid treatment). However, the mechanism of mTOR participation in TLE is largely unknown. Therefore, the aims of our team are to identify mTOR targets during aberrant plasticity and establish the potential of mTOR inhibitors as anti-epileptic drugs. We first determined the pattern of mTOR activity and the pattern of activity of its best known targets during kainic acid-evoked seizures. Biochemical and immunohistochemical analysis revealed a bimodal character of mTOR activation, which increased 2 h post-kainic acid injection and again 24 h later. The strongest activation of mTOR and its downstream effector S6 was observed in the cortex, hippocampal formation, and amygdala. We also noted differences in subcellular mTOR distribution upon kainic acid injection, namely its strong activation in the nucleus. Interestingly, we were able to show, for the first time, a switch in cell specificity of S6 activation. At early times after kainic acid administration (up to 24 h), S6 activation was observed mostly in neurons. Afterward (up to 72 h), mainly glial cells were characterized by increased phospho-S6 immunoreactivity (Fig. 3). We next tested whether organotypic cultures of the hippocampus may serve as a good

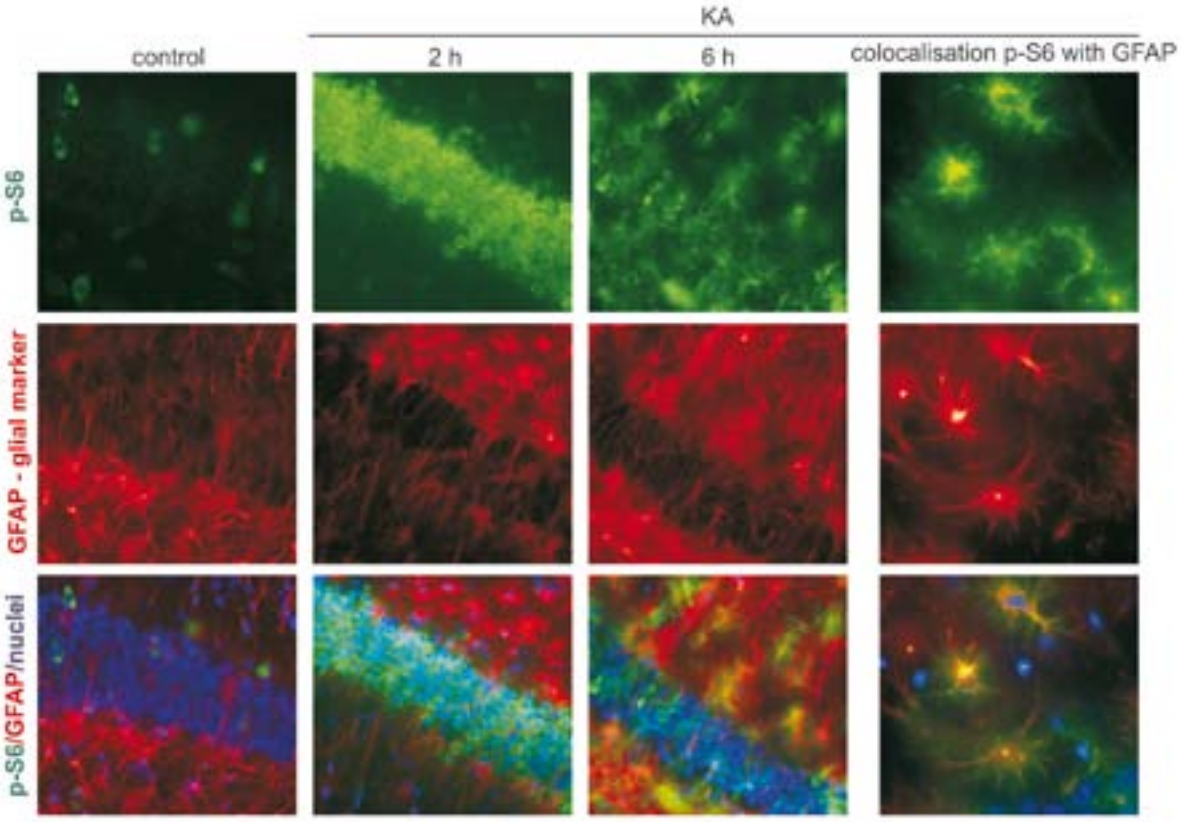


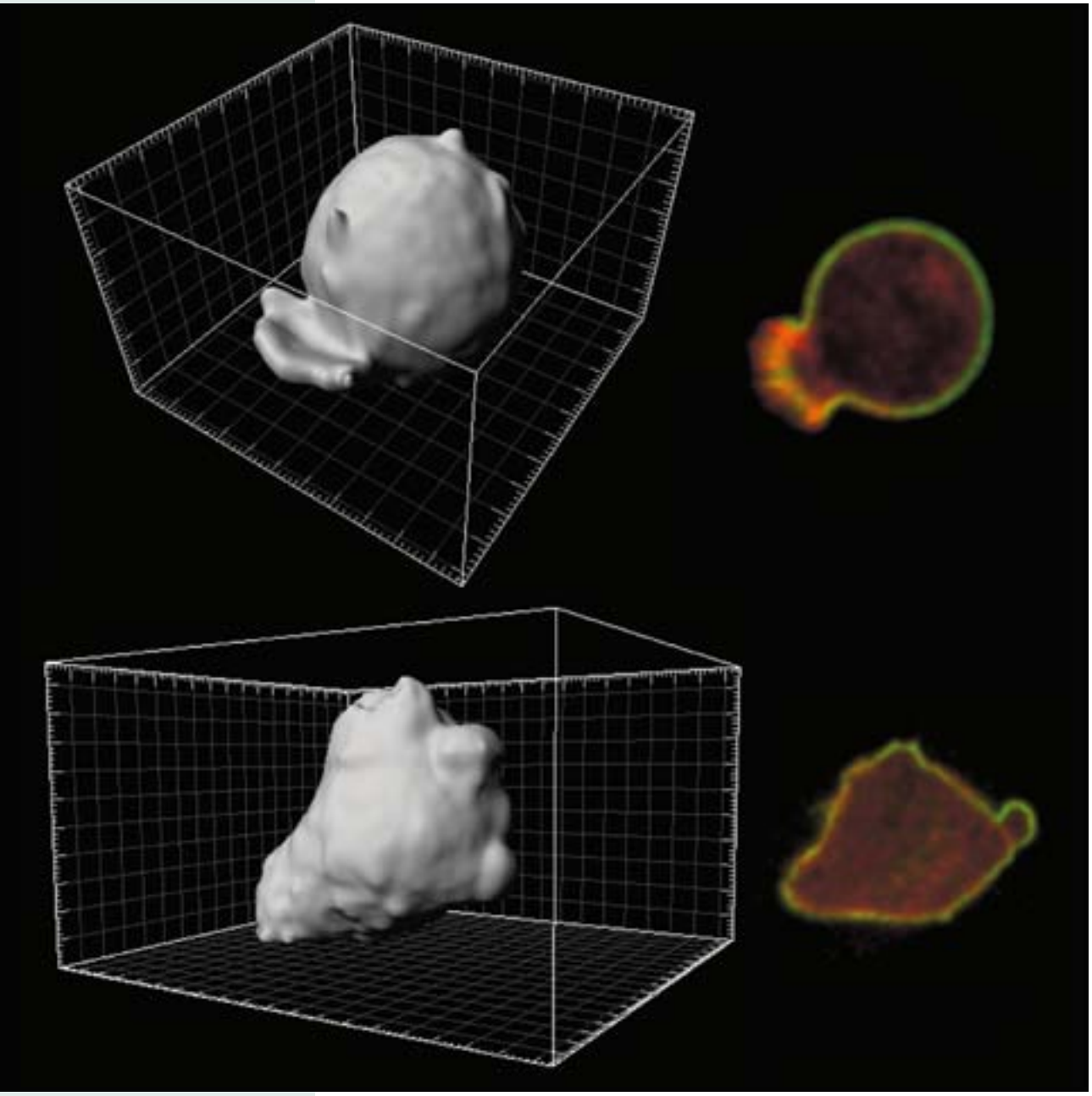
Fig. 3. Seizures induced by kainic acid application in rats resulted in an increase in mTOR pathway activity (S6 protein phosphorylation), but with different kinetics depending on cell type. Neurons responded as early as 2 h while reactive astrocytes (highlighted by immunofluorescence against GFAP) turned on mTOR response later (author: Matylda Macias).

model of kainic acid-induced events during epileptic seizures. Indeed, application of kainic acid to organotypic cultures induced, similar to the in vivo situation, phosphorylation of mTOR, p70S6K, and S6 and expression of c-fos and EAAT1 genes. Rapamycin blocked the effects of kainic acid (with the exception of c-fos which is not an mTOR target). These results proved that the dynamics of molecular events are similar in organotypic cultures and in vivo. Using this model, we have begun to identify mTOR downstream effectors in kainic acid-treated slices with microarray technology following application of an mTOR inhibitor (work done in collaboration with Prof. Przewlocki, Institute of Pharmacology, Cracow). Although this analysis confirmed a known strong effect of kainic acid on gene expression, we were additionally able to identify two groups of genes, the kainic acid-induced expression of which was influenced by mTOR inhibition. The expression of genes belonging to the first group containing mostly immediate early genes (IEGs) was potentiated by Rapamycin. This observation is consistent with previous reports of the potentiating effects of protein inhibitors on IEG levels. The second group, however, contains several unrelated genes, whose kainic acid-induced expression is prematurely terminated by Rapamycin. Rapamycin was recently shown to prevent anatomical changes induced by seizures, and the future direction for this project is to identify the mTOR targets induced by epileptic drugs that contribute to morphological changes in neuronal circuitry and underlie epileptogenesis.

A major research effort of our second new project, conducted under the auspices of the 7FP NeuroGSK3 consortium, is to answer the fundamental physiological and pathological questions surrounding GSK3 kinases in the central nervous system. Our laboratory is interested in understanding the molecular mechanisms of physiological and pathological changes of neuronal morphology, within the framework of a NeuroGSK3 grant, by focusing on the role of GSK3 and its upstream and downstream targets in shaping dendritic arbors and dendritic spines, both under normal conditions as well as in Alzheimer's disease pathology. mTOR has been described in different model systems to be an either upstream regulator or a downstream target of GSK3. Increased activity of one of these kinases is usually associated with a decrease in activity of the other. Interestingly, activities of both mTOR and GSK3 were reported to be increased in animal models of Alzheimer's disease and in brain tissue of Alzheimer's patients, suggesting an interplay between the impairment in those two signaling pathways in this pathology. Moreover, additional crosstalk between GSK3 and mTOR occurs at the level of their downstream targets, such as the Tau protein, one of the major suspects in Alzheimer's disease progression. Also important is the fact that both dendritic arbors and spines are impaired in the brains of Alzheimer's disease patients and animal models of Alzheimer's disease. Based on these premises, we sought to understand the reciprocal regulation of mTOR and GSK3 in neurons under physiological and pathological conditions and verify the importance of this crosstalk for the regulation of neuronal morphology. Using mouse cortical neuron cultures, we found that GSK3 is an inhibitor of mTOR in mature neurons. Interestingly, in developing neurons, we observed that GSK3 might also act downstream of mTOR, depending on the availability of proper trophic support. Moreover, we found that several conditions mimicking synaptic or homeostatic plasticity change GSK3 and mTOR activity in opposite directions. For example, treatment with 100 mM NMDA for 3 min, mimicking long-term synaptic depression, causes an increase and a decrease in GSK3 and mTOR activity, respectively, 3 h later. The converse was obtained in the case of BDNF treatment which should lead to a protein translation-dependent increase in synaptic efficacy. We are now intensively investigating whether GSK3 still controls mTOR activity or whether the crosstalk between these two is lost under these plasticity-mimicking conditions. As a next step we plan to perform an identical stimulation design but with the use of biAT transgenic mice carrying mutated Tau and β-amyloid to determine disturbances in crosstalk.

Our research plans for 2010 include:

- Further investigation of mTOR-dependent microtubule dynamics regulation.
- Further research on genes identified in shRNA screens and with the use of microarrays in the context of dendritogenesis, TSC development, and epilepsy. In collaboration with Dr. Johen Herms (Munich) and Dr. Ype Elgersma (Rotterdam), we are seeking to verify the relevance of our findings in in vivo animal models.
- Initiation of proteomic screens for mTOR-interacting partners in neurons under pathological (epilepsy) conditions.
- Investigation of reciprocal regulation loops between mTOR and GSK3 under Alzheimer's disease-mimicking conditions and its importance for neuronal morphological changes.



Jurkat cell forming a lamellipodium (top) and Walker carcinosarcoma cell forming blebs (bottom). Cells were transfected with LifeAct-mCherry (actin-marker) and EGFP-CAAX (membrane-marker). Surface rendering (left) was done with Imaris software based on the membrane labelling. (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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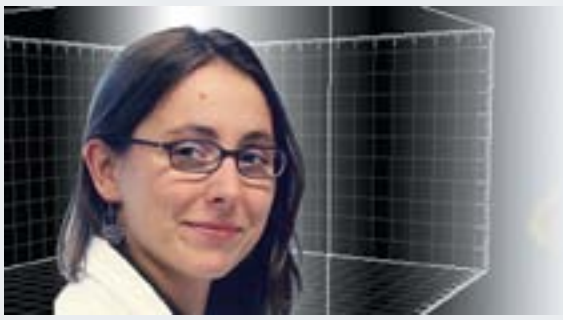
Julia Roensch, BSc

MAX-PLANCK-GESELLSCHAFT

INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY

The equipment and running costs for the lab, including personnel, are provided by IIMCB (through research grants financed by the Polish Ministry of Science and Higher Education).



Ewa Paluch
PhD

DEGREES

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

RESEARCH TRAINING

2001-2005	PhD studies at the Institut Curie, Paris, France
2000-2001	DEA (equivalent Masters) research project in Biophysics, Institut Curie, Paris, France
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

since 2006	Joint MPI-CBG/PAN group leader at IIMCB, located at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden
2005	Scientist position at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

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 from the Ligue Nationale contre le Cancer, France
2001-2004	PhD scholarship from CNRS, France
2000	Agrégation in Physics (French national competition, rank: 6th)
1998-2001	full salary from the 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

Selected publications

- Tinevez JY, Schulze U, Salbreux G, Roensch J, Joanny JF, **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
- *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.

*Papers marked with an asterisk do not have the IIMCB affiliation of the authors

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)

Grants

- 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); 4 692 929 PLN; 2009-2012
- *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); 70 600 euros + 1 phD position / team; 2009-2012
- *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; 2008-2011

- Polish-German Special Grant "The role of cell cortex contractility in the establishment and positioning of the cleavage furrow", (JRGP/37/2005) The Max Planck Society (MPG) – the Polish Academy of Sciences (PAN) MPI-CBG Junior Research Program – Laboratory of Cortex Movement and Cell Division MGP/PAN; 3,024,200 PLN; 2006-2009

*Grants marked with an asterisk are not affiliated to the IIMCB

Research

The main goal of the group's research is to understand how the mechanical properties of the cell are regulated at the protein level to achieve controlled cellular deformations. We particularly focus on the cell cortex, a network of actin, myosin, and associated proteins that lies beneath the plasma membrane and determines the shape of the cell body. The cortex enables the cell to resist externally applied forces and to exert mechanical work. As such, it plays a role in normal physiology during events involving cell deformation, such as mitosis, cytokinesis, and cell locomotion, and in the pathophysiology of diseases such as cancer in which cortical contractility is often upregulated. Despite its importance, very little is known about how the cortex is assembled and regulated.

The biological function of the cortex relies on its ability to contract and exert forces. As such, the cortex is an intrinsically mechanical structure, and its biological properties cannot be understood in isolation from its mechanics. 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, 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 roles are still poorly understood. We investigate the physical and biological mechanisms of bleb formation and study their function during cytokinesis and migration. Moreover, de novo cortex assembly can be observed at the bleb membrane, and we use blebs as tools for the study of cortex nucleation and growth.

The staff, composed of biologists and physicists, combines biophysical and molecular approaches. Our main lines of research are the following:

1. Regulation of cortex assembly and cortex mechanics

We seek to characterize the role of various cortical components in cortex mechanics. To achieve this aim, we study the effects of depletion of target cortical proteins on (i) cortex mechanical properties and (ii) the dynamics of cortex assembly.

We have developed experimental assays to measure cortical tension and turnover. Using a micropipette aspiration setup, we showed that cortex tension depends not only on the activity of myosin motors, but also on the level of proteins involved in actin turnover (Tinevez et al., Proc Natl Acad Sci USA, 2009). We are currently analyzing the influence of the different myosin motors present in the cortex on cortical tension and turnover. We are also working on designing methods to measure cortex thickness (using STED microscopy) and elastic modulus (with an optical stretching device).

To study the regulation of cortex growth, we developed an assay for semiautomatic analysis of cortex assembly. We use cultured cells blocked in metaphase, in which the cortical layer is particularly prominent, and induce blebs in a controlled manner using laser ablation of the cortex. The bleb membrane is initially devoid of cortex and reassembles one within a few minutes following bleb induction. Recruitment of actin and myosin to the bleb membrane is then quantified with automated software. We have begun analyzing the influence of the depletion of cortical components on cortex reassembly and are seeking to identify the proteins essential for the nucleation and growth of the cortical network.

Very little is known about the composition of the actin cortex. To narrow down the list of potential target genes for the assays described above, we are seeking to determine the composition of the cortices of isolated blebs. In collaboration with Guillaume Charras (UCL, London) and Philippe Roux (IRIC, Montreal; partner groups in an HFSP Young Investigator Grant; see grants list above), we have developed a method to isolate blebs from mammalian cells. The group of Philippe Roux is currently analyzing the proteic composition of the isolated blebs using mass spectrometry. Preliminary results have provided a short-list of actin nucleators, and their influence on cortex assembly is currently being tested in our lab.

2. Mechanisms of bleb formation

The growth of blebs depends on myosin activity and is commonly believed to directly result from intracellular pressure, but this hypothesis had not been directly tested, and the mechanisms of bleb growth remain elusive.

Using laser ablation of the cortex to induce bleb formation, we have shown that bleb growth is driven by, and considerably reduces, intracellular pressure (Tinevez et al., Proc Natl Acad Sci USA, 2009). By inducing blebs on cells with different tensions, we subsequently demonstrated the existence of a critical tension below which a bleb cannot expand. Above this threshold, the size of a bleb strongly depends on tension, and this dependence can be fitted to a theoretical model of the cortex, yielding an estimation of cellular elastic parameters (collaboration with the group of J.F. Joanny, Institut Curie, Paris).

We are now analyzing the dynamics of bleb expansion to elucidate which mechanical dissipation sources are the major limiting factors for bleb expansion. Indeed, the timescale and rate of bleb expansion can be limited by the flow of plasma membrane or the flow of cytoplasm into the growing bleb. By quantitatively analyzing the dynamics

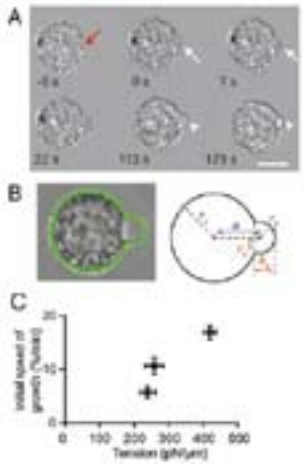


Fig. 1: (A) Timelapse of a laser ablation experiment. After ablation (0s, red arrow), a bleb expands (white arrows) and retracts (white arrowheads) within a few minutes. (B) Image analysis. The cell contour is fitted with two intersecting circles. (C) Bleb expansion dynamics. Initial speed of bleb growth as a function of cortical tension (modified from Tinevez et al., Proc Natl Acad Sci USA, 2009).

of bleb growth (Fig. 1) and fitting them to physical models (Tinevez et al., Proc Natl Acad Sci USA, 2009), we seek to test to what extent dissipation attributable to each of these flows limits bleb expansion. This is particularly important because the type of protrusion formed by a cell is likely to affect its migration pattern (see section 4 below). Modifying the magnitude of dissipation linked to bleb growth can thus be used by cells to favor or reduce the formation of blebs vs. other protrusion types, such as lamellipodia.

3. Role of cortex tension and blebs during cytokinesis

The formation and ingression of the cleavage furrow during cytokinesis relies on a controlled reorganization of the actin cortex. Although most studies of cytokinesis focus on the contractile ring at the cell's equator, a significant amount of actin and myosin is also present at the poles of a dividing cell. We discovered that disruption of this polar cortex by laser ablation or local drug delivery leads to oscillations of the cleavage furrow and results in division failure. Similar furrow oscillations could be induced by depletion of different actin binding proteins. In all cases, oscillations appeared to be triggered by an imbalance in cortical contractility between the two poles of the dividing cell. Strikingly, we could also observe small oscillations of the furrow in control divisions, although their amplitude remained limited, allowing for division to proceed (Fig. 2). Based on our observations, we have proposed that the cell tightly controls the contractility and stability of the polar cortex during furrow ingression. Under control conditions, any imbalance in contractile forces leads to partial disassembly of the cortex and to the formation of blebs, which buffer intracellular pressure. When the polar cortex is stabilized or when a significant contractility imbalance between the two poles is introduced, the cleavage becomes unstable and cytokinesis fails.

We further tested this mechanism by inhibiting bleb formation during cytokinesis using lectins that cross-link the cortex from the outside and limit membrane deformations, or by inducing further bleb formation using laser ablation. We found that reducing bleb formation triggers cell shape oscillations, whereas inducing bleb formation in oscillating cells interferes with oscillations. Altogether, our results support the hypothesis that blebs act as pressure buffers, preventing the build-up of shape instabilities between the two poles of a dividing cell.

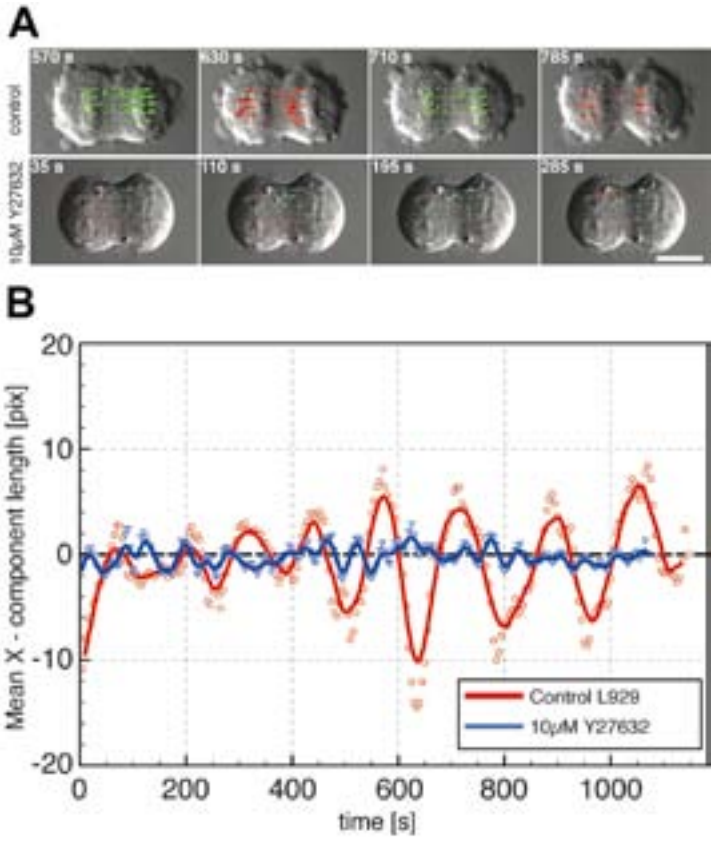


Fig. 2: Small shape oscillations in dividing L929 cells. (A) Image sequence from a control dividing cell and from a dividing cell in which contractility has been reduced by treatment with Y27632, a Rho-kinase inhibitor. Arrows indicate the magnitude of cytoplasmic flows in the cells (red arrows are oriented toward the right, and green arrows toward the left). (B) Mean amplitude of the cytoplasmic flows (from panel A, projected on the horizontal axis) over time. The control cell (blue line) displays oscillating cytoplasmic flows (authors: Jakub Sedziński, Maté Biro).

4. Protrusion formation during migration in three-dimensional environments

In three-dimensional environments, bleb-based migration is a widespread alternative to lamellipodial migration and is commonly used by cancer cells and during development (Charras and Paluch, Nat Rev Mol Cell Biol, 2008). It is not known why cells form one or the other type of protrusion or how the cells can switch between protrusion types. Strikingly, certain cell types (e.g., mesendodermal cells in Danio rerio [zebrafish] embryos) are able to form both lamellipodia and blebs at the same time. We have initiated a study of the mechanisms of formation of these protrusions and of their respective contributions to cell migration in the zebrafish embryo (collaboration with the C.P. Heisenberg laboratory, MPI-CBG). We characterized wildtype migration and showed that the protrusions formed by mesendodermal progenitors consist of blebs, lamellipodia, and filopodia. We also showed that the expression of dominant negative (respectively constitutively active) ezrin (a protein linking the actin cortex to the membrane) shifts this distribution and leads to the formation of more (respectively fewer) blebs. Interestingly, both treatments led to migration defects. Increasing bleb formation slowed down migration by reducing the directional persistence of the migrating cells, whereas decreasing bleb formation reduced the instantaneous speed of the cells, without affecting their directionality. Altogether, these results indicate that blebs may allow for rapid exploration of the environment, with less directional focus than lamellipodia formation.

We are currently extending these studies to an in vitro culture system. We use Walker carcinosarcoma cells, which can be induced to form blebs or lamellipodia exclusively, depending on culture conditions (Fig. 3). We have initiated a mechanical comparison of the two sublines and showed that cortical tension is lower in the subline forming lamellipodia than in the subline forming blebs. We also performed a microarray analysis of gene expression levels in the two sublines and are currently testing the role of target proteins in protrusion formation.

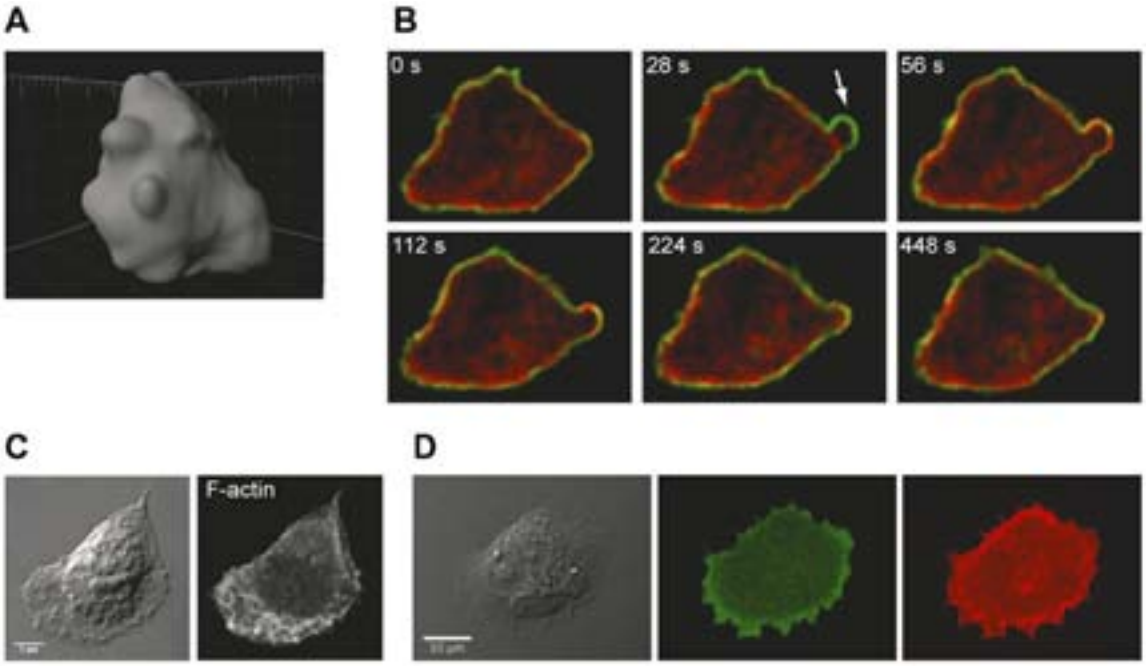
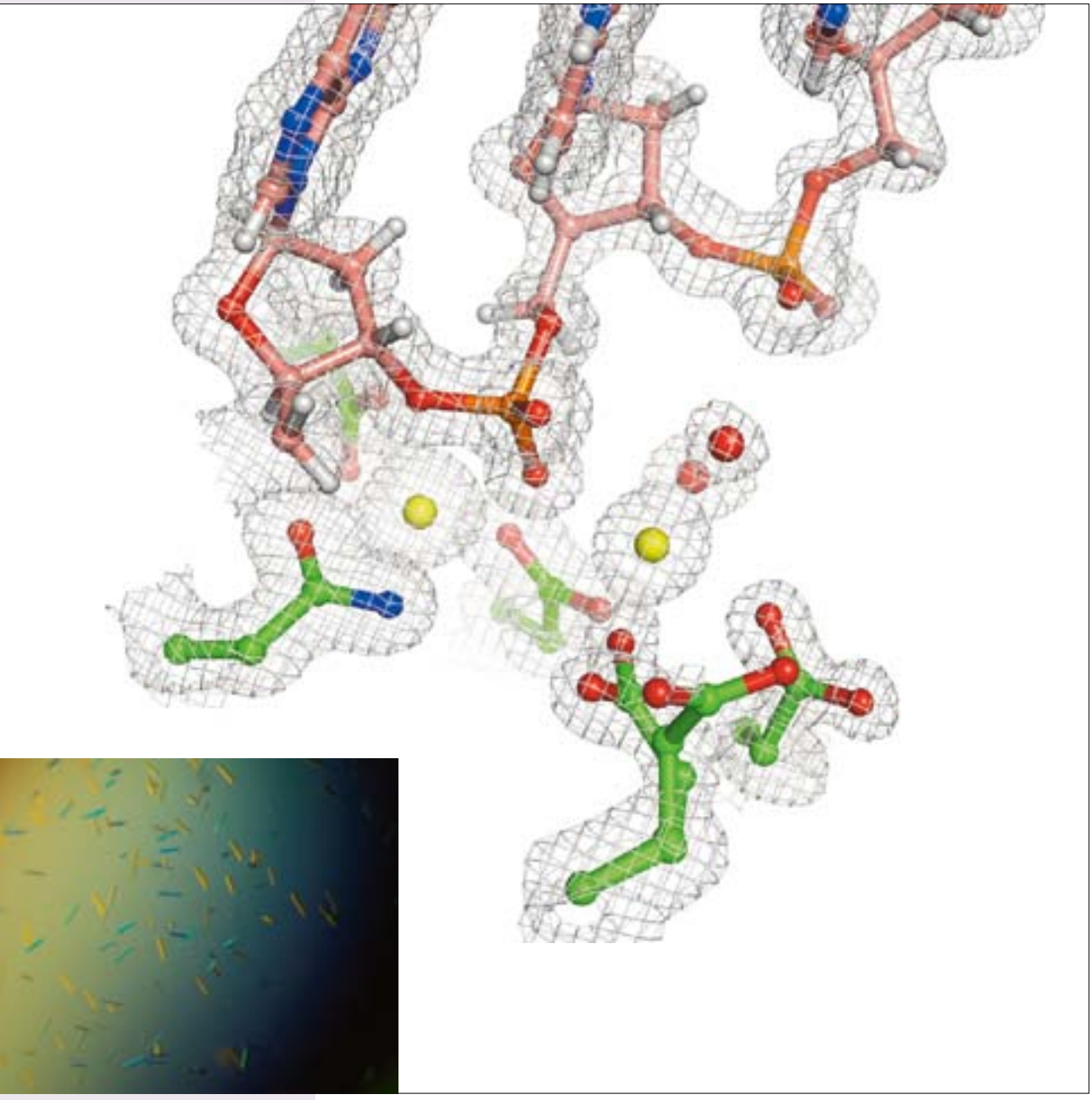


Fig. 3: Protrusions in the Walker carcinosarcoma sublines. (A) Surface reconstruction of a cell from the blebbing subline. Ball-like, spherical protrusions at the leading edge indicate blebs. (B) Timelapse picture series of blebbing cell. Green, membrane (EGFP-CAAX); red, F-actin (LifeAct-mCherry); arrow, membrane separated from actin cortex. (C) Fixed cell from the lamellipodial subline showing actin filaments in the lamellipodium visualized with rhodamine-phalloidine. (D) Live cell from the lamellipodial subline expressing EGFP-CAAX (green) and LifeAct-mCherry (red). The protrusions are actin-filled membrane sheets, characteristic of lamellipodia (author: Martin Bergert).



Crystals of bacterial RNase H2 in complex with nucleic acid substrate (author: Monika Rychlik).

Structure of RNase H1 in complex with RNA/DNA hybrid solved at 1 Å resolution. The carboxylates forming the active site are shown in green and the RNA in pink. Two calcium ions observed at the active site are shown in yellow. Water molecules are shown in red, one of which is the attacking nucleophile critical for the reaction (author: Monika Rychlik, Marcin Nowotny).

Laboratory of **Protein Structure**

Lab Leader:

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Post-doctoral Fellows:

Elżbieta Nowak, PhD

Karolina Górecka, PhD

Junior Researchers:

Małgorzata Figiel, MSc

Marcin Jaciuk, MSc

Jakub Jurkowski, MSc

Mirosław Śmietański, MSc

Lab Manager:

Monika Rychlik, MSc

Technician:

Jadwiga Dyttus

Marcin Nowotny, PhD

DEGREES

- PhD *magna cum laude* in biochemistry, Nencki Institute of Experimental Biology PAN, Department of Molecular and Cellular Neurobiology, Warsaw, 2002
- MSc in organic chemistry and biochemistry, Warsaw University, Department of Chemistry 1998

POSTDOCTORAL TRAINING

2003-2008 Postdoctoral Fellow, Wei Yang laboratory, National Institutes of Health, National Institute of Diabetes & Digestive & Kidney Diseases, Bethesda MD, USA

PROFESSIONAL EMPLOYMENT

Since 2008 Head of the Laboratory of Protein Structure, IIMCB

HONORS, PRIZES, AWARDS

2003 Prime Minister's award for PhD thesis

2001, 2002 Annual Stipend for Young Scientists of the Foundation for Polish Science

1999 Fellowship of the Kronenberg Bank Foundation

Selected publications

- **Nowotny M**. Retroviral integrase superfamily: the structural perspective (review). EMBO Rep, 2009; 10:144-51
- **Nowotny M**, Yang W. Structural and functional modules in RNA interference. Curr Opin Struct Biol. 2009;19:286-293. Review
- ***Nowotny M**, Cerritelli SM, Ghirlando R, Gaidamakov SA, Crouch RJ, Yang W. Specific recognition of RNA/DNA hybrid and enhancement of human RNase H1 activity by HBD. EMBO J, 2008; 27:1172-81
- ***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
- ***Nowotny M**, Yang W. Stepwise analyses of metal Ions in RNase H catalysis: From substrate destabilization to product release. EMBO J, 2006; 25:1924-33
- *Yang W, Lee JY, **Nowotny M**. Making and Breaking Nucleic Acids: Two-Mg2+-ion Catalysis and Substrate Specificity, (review). Mol Cell, 2006; 22:5-13
- ***Nowotny M**, Gaidamakov SA, Crouch RJ, Yang W. Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. Cell, 2005; 121:1005-16
- *Lee YT, Jacob J, Michowski W, **Nowotny M**, Kuznicki J, Chazin WJ. Human Sgt1 binds HSP90 through the CHORDSgt1 domain and not the tetratricopeptide repeat domain. J Biol Chem, 2004; 279:16511-7
- ***Nowotny M**, Spiechowicz M, Jastrzebska B, Filipek A, Kitagawa K, Kuznicki J. Calcium-regulated interaction of Sgt1 with S100A6 (calcyclin) and other S100 proteins. J Biol Chem, 2003; 278:26923-8
- *Filipek A, Jastrzebska B, Nowotny M, Kuznicki J. CacyBP/SIP, a calcyclin and Siah-1-interacting protein, binds EFhand proteins of the S100 family. J Biol Chem, 2002; 277:28848-52
- *Filipek A, Jastrzebska B, **Nowotny M**, Kwiatkowska K, Hetman M, Surmacz L, Wyroba E, Kuznicki J. Ca2+-dependent translocation of the calcyclin-binding protein in neurons and neuroblastoma NB-2a cells. J Biol Chem, 2002; 277:21103-9
- ***Nowotny M**, Bhattacharya S, Filipek A, Krezel AM, Chazin W, Kuznicki J. Characterization of the interaction of calcyclin (S100A6) and calcyclin-binding protein. J Biol Chem, 2000; 275:31178-82.

*Papers marked with an asterisk have no the IIMCB affiliation of the authors

Description of Current Research

Our laboratory focuses on structural and biochemical studies of nucleic acid enzymes. Our primary method is protein crystallography. Our projects can be subdivided into three groups:

1. Structural studies of substrate complexes of members of the Retroviral Integrase Superfamily.
2. Structural studies of reverse transcriptases.
3. Structural studies of UvrA DNA repair protein.

1. Retroviral Integrase superfamily

The Retroviral Integrase superfamily (RISF) comprises an important and interesting nucleic acid processing enzyme family, containing transposases, integrases, and various nucleases. They are involved in a wide range of processes, such as transposition, replication, DNA repair, homologous recombination, and the action of siRNAs. One of the best-characterized members of RISF is RNase H, which is a nuclease that binds RNA/DNA hybrids in a sequence nonspecific manner and degrades the RNA strand. Two types of RNases H have been identified: type 1 (RNase H1) and type 2 (RNase H2). Type 1 enzymes are present in all forms of life, from bacteria to animals. They are also an integral part of reverse transcriptases. In HIV reverse transcriptase, RNase H activity is essential for viral progression and is also one of the least explored and most promising drug targets for the treatment of AIDS. Substrate complex structures of type 1 RNases H revealed the mechanism of RNA/DNA recognition and demonstrated that the catalysis relies on two metal ions (Nowotny et al., Cell, 2005; Nowotny et al., Mol Cell, 2007). To better understand the catalytic mechanism of RNase H1, we solved its ultra-high resolution structure in complex with the substrate at 1 Å resolution, and we plan to extend these studies with neutron diffraction experiments. The two approaches will allow us to directly observe the protonation of the active site of RNase H1, which so far has not been achieved for any nuclease in complex with nucleic acid.

Members of RISF share the same fold of the catalytic core and very similar architecture of the active site, yet they act on a wide range of nucleic acids. For example, RNase H2 can cleave single ribonucleotides embedded in DNA and is therefore hypothesized to participate in DNA repair. We solved the crystal structures of bacterial type 2 RNase H in complex with double-stranded DNA with a single ribonucleotide present in one of the strands. The structures revealed a unique mechanism in which the RNA-DNA junction is specifically deformed upon binding to the protein. This deformation allows the phosphate backbone of the substrate to participate in metal ion coordination at the active site. Therefore, substrate recognition and catalysis are coupled to achieve high cleavage specificity.

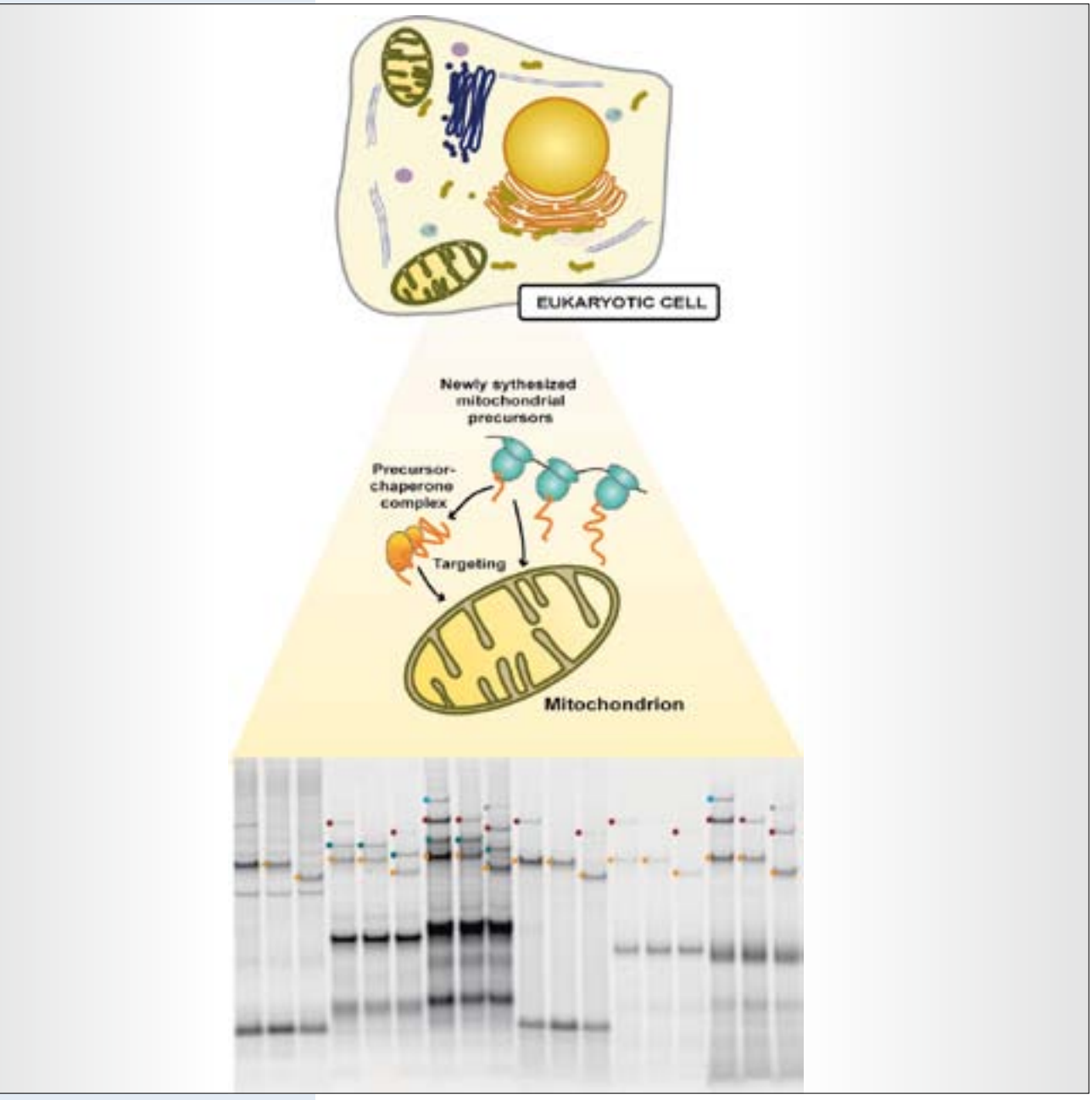
Human RNase H2 is a complex of three proteins (i.e., the catalytic subunit and two auxiliary subunits). Mutations of this enzyme result in Aicardi-Goutieres syndrome (AGS), an autosomal recessive genetic disorder with symptoms similar to in utero viral infection which severely affects the nervous system. The human enzyme is thus essential, but no structural information about it has been available. We solved a 3.1 Å structure of the human RNase H2 complex. The structure revealed that the auxiliary subunits bind to the catalytic subunit away from the substrate binding interface and are therefore unlikely to participate in nucleic acid binding. The structure of the auxiliary subunits resembles TFIIF transcription factor, suggesting that their role is to couple RNase H2 action to transcription.

2. Reverse transcriptases

Reverse transcriptases are multifunctional enzymes catalyzing the conversion of single-stranded RNA to dsDNA. This process is essential for the life cycle of certain viruses (e.g., retroviruses such as HIV or hepadnaviruses such as hepatitis B). The crystal structures of only two reverse transcriptases have been solved. Only structures of complexes of HIV RT with nucleic acids are available. Significant variability exists in RT architecture between different viruses, and several important aspects of the mechanism of RT action remain unclear (e.g., the way in which the polymerase and RNase H activities are coordinated). No structural information is available for hepatitis B virus RT (HBV RT), which is an important drug target. This enzyme cannot be produced in an active form in sufficient quantities to allow structural studies. Therefore, we use bioinformatics to identify its close homologs, crystallize them, and subsequently solve their structures. Based on these structures, an accurate homology model of HBV RT will be built. We have also undertaken co-crystallization experiments of these new RTs with their nucleic acid substrates. We are seeking to identify proteins that will readily form crystals with various nucleic acids corresponding to particular stages of reverse transcription. These snapshots will allow us to reconstruct the detailed mechanism of the reaction.

3. Structural and biochemical studies of UvrA DNA repair protein

DNA molecules, the carriers of genetic information, are susceptible to chemical damage. One of the primary pathways to remove these modifications is nucleotide excision repair (NER), in which a stretch of bases harboring the lesion is cleaved out and the resulting gap is filled by a DNA polymerase. The remarkable feature of NER is the fact that it can act on a wide spectrum of unrelated DNA lesions, varying greatly in chemical structure. In bacteria, one of its key components is UvrA protein, which is hypothesized to be the first to detect DNA damage. It then recruits other components of NER. Recently, a crystal structure of apo UvrA has been reported, but detailed information about the mechanism of damaged DNA recognition is still lacking. By solving a crystal structure of UvrA with different types of damaged DNA, we would like to learn how the remarkably wide specificity of the NER system is achieved. We hope to reveal which features of different lesions are used by UvrA to recognize the damage. The enzyme contains two ATPase domains, and ATP hydrolysis is essential for damage recognition. Co-crystallization of UvrA with ATP analogs, ADP, and without the nucleotide should reveal the conformational changes during ATP hydrolysis and their consequences for DNA binding. These studies should help explain the central question in DNA repair, namely the mechanisms of damage recognition.



Authors: Dusanka Milenkovic, Judith Müller and Agnieszka Chacińska

Laboratory of **Mitochondrial Biogenesis**

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Junior Researchers:

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Agnieszka Górnicka, MSc

Aksana Varabyova, MSc

Lidia Wróbel, MSc

Research Assistant:

Anita Chodkowska, MSc

Undergraduate Students:

Paulina Kwiatkowska

Inmaculada Mora Espi

Kamila Ornoch

Agnieszka Chacińska,
PhD, DSc. Habil.

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	Master Degree in Molecular Biology, University of Warsaw
1988-1993	Biology, University of Warsaw, Poland

AWARDS

2010	EMBO Installation Grantee
2009	Laureate of Welcome Programme – Foundation for Polish Science
2008	Eugen-Graetz Prize for Research, University of Freiburg
2001-2003	Long-term FEBS fellowship
2001	Award for the PhD thesis, Institute of Biochemistry and Biophysics, Warsaw
1997	Grant for young scientists from the Polish State Committee for Scientific Research
1996	Short-term FEBS fellowship

RESEARCH EXPERIENCE AND APPOINTMENTS:

Since 2009	Professor and Leader of the Laboratory of Mitochondrial Biogenesis, International Institute of Molecular and Cell Biology in Warsaw
2008-2009	Associate Member of Excellence Cluster BIOSS–Centre for Biological Signalling Studies, University of Freiburg
2007-2009	Member of the Board, Collaborative Research Centre (SFB 746)
2007-2010	Project Leader in the Collaborative Research Centre (SFB 746)
2004-2009	Group Leader (German equivalent of Assistant Professor), Institute for Biochemistry and Molecular Biology, University of Freiburg
2001-2004	Postdoctoral Fellow in the laboratory of Prof. Nikolaus Pfanner, University of Freiburg, Germany
1999	Visiting scientist in the laboratory of Prof. Sabine Rospert, Max Planck Research Unit, Halle, Germany
1997	Visiting scientist in the laboratory of Prof. Gottfried Schatz, Biozentrum, University of Basel, Switzerland
1994-2000	Doctoral research, Institute of Biochemistry and Biophysics, Warsaw, with Prof. Magdalena Boguta.

Selected publications

- **Chacinska A**, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. Importing mitochondrial proteins: machineries and mechanisms. Cell, 2009; 138:628-644
- Milenkovic D, Ramming T, Müller JM, Wenz LS, Gebert N, Schulze-Specking A, Stojanovski D, Rospert S, **Chacinska A**. Identification of the signal directing Tim9 and Tim10 into the intermembrane space of mitochondria. Mol Biol Cell, 2009; 20:2530-9.
- **Chacinska*** **A**, Guiard* B, Müller JM, Schulze-Specking A, Gabriel K, Kutik S, Pfanner N. Mitochondrial biogenesis: switching the sorting pathways of the intermembrane space receptor Mia40. J Biol Chem, 2008; 283:29723-9. (*equal contribution)
- Stojanovski D, Milenkovic D, Müller JM, Gabriel K, Schulze-Specking A, Baker MJ, Ryan MT, Guiard B, Pfanner N, **Chacinska A**. Mitochondrial protein import: precursor oxidation in a ternary complex with disulfide carrier and sulfhydryl oxidase. J Cell Biol, 2008; 183:195-202
- Müller JM, Milenkovic D, Guiard B, Pfanner N, **Chacinska A**. Precursor oxidation by Mia40 and Erv1 promotes vectorial transport of proteins into the mitochondrial intermembrane space. Mol Biol Cell, 2008; 19:226-236
- Milenkovic D, Gabriel K, Guiard B, Schulze-Specking A, Pfanner N, **Chacinska A**. Biogenesis of the essential Tim9-Tim10 chaperone complex of mitochondria: site-specific recognition of cysteine residues by the intermemembrane space receptor Mia40. J Biol Chem, 2007; 282:22472-80
- **Chacinska A***, Lind M*, Frazier AE, Dudek J, Meisinger C, Geissler A, Sickmann A, Meyer HE, Truscott KN, Guiard B, Pfanner N, Rehling P. Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17. Cell, 2005; 120:817-829 (*equal contribution)
- Rissler M, Wiedemann N, Pfannschmidt S, Gabriel K, Guiard B, Pfanner N, **Chacinska A**. The essential mitochondrial protein Erv1 cooperates with Mia40 in biogenesis of intermembrane space proteins. J Mol Biol, 2005; 353:485-492
- **Chacinska A**, Pfannschmidt S, Wiedemann N, Kozjak V, Sanjuán Szklarz LK, Schulze-Specking A, Truscott KN, Guiard B, Meisinger C, Pfanner N. Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins. EMBO J, 2004; 23:3735-46

All papers have no IIMCB affiliation.

Description of Current Research

Mitochondria play an important role in cellular metabolism and regulatory processes, such as apoptosis. Thus, the formation of functional mitochondria is essential for cellular function in the entire eukaryotic kingdom, from unicellular organisms to mammals. Mitochondria comprise 1000-1500 cellular proteins, which are synthesized outside of the mitochondria in the cytosol. Thus, the biogenesis of mitochondria relies on the efficient import, sorting, and maturation of proteins governed by the conserved protein translocases and other complex machineries. In the course of our work with Prof. Nikolaus Pfanner 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 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 lacking a classical mitochondrial leader sequence.

Our current research, supported by a Welcome grant from the Foundation for Polish Science, an EMBO Installation grant, and a grant from the Ministry of Science and Higher Education, will explore novel and exciting links between disulfide bond formation mechanisms and mitochondrial protein homeostasis. We postulate the presence of unique mechanisms involved in protein biogenesis that involve crosstalk between the cytosol and mitochondrial compartments. Our research addresses three major and related issues:

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

Our goal is to better understand the complex and dynamic processes involved in the formation of functional organelles, the maintenance of mitochondrial protein homeostasis, and their failure resulting in pathology.

Educational Activities

IIMCB continues its doctoral program in partnership with other research and educational institutes of the Ochota Campus. Currently 37 PhD students are on board within the doctoral programs of the Institute of Biochemistry and Biophysics, of the Nencki Institute, of the University of Warsaw, of the Postgraduate School of Molecular Medicine (SMM) and of the Foundation for Polish Science (FNP). The international PhD program run in collaboration with Utrecht University has entered the last phase: currently, four students are still enrolled in this program. Additionally, IIMCB is in the process of recruiting a group of seven students within the International PhD Program in Molecular Biology, run together with the Institute of Biochemistry and Biophysics, based on FNP grant "PhD Programme in Molecular Biology: Studies of nucleic acids and proteins - from basic to applied research".

Postgraduate School of Molecular Medicine (www.iimcb.gov.pl/smm.php)

Medical Universities in Warsaw, Gdańsk, Wroclaw, Łódź, Lublin as well as International Institute of Molecular and Cell Biology, Nencki Institute of Experimental Biology, Institute of Biochemistry and Biophysics, Mossakowski Medical Research Centre, Oncology Centre-Institute and the Foundation for Experimental and Clinical Oncology have jointly founded the Postgraduate School of Molecular Medicine (SMM). The main goal of the School is to offer a new postgraduate PhD program in the field of molecular medicine, which is addressed to medical, biology and pharmacology postgraduate students in Poland. Since 2002, SMM has been opened to foreign students. SMM is formally affiliated with Medical University of Warsaw, which is responsible for administration of the school. According to its by-laws, the School is managed by Director and Scientific Council elected by all founding institutions. SMM admits students (up to twelve per year) for the four-year doctoral program. The candidates are requested to present a scientific program of their doctoral research, the scientific merit of which is carefully evaluated by the Recruitment Committee of SMM, as well as by independent reviewers in Poland and from abroad. Ten groups of students were accepted during the period of 1998-2010, including eight foreign individuals. Successful candidates accomplish their scientific program, under supervision of their tutors, in home laboratories throughout Poland. Members of SMM Scientific Council evaluate students' progress annually. The tutorial program offered to students includes theoretical (lectures and seminars) and practical courses (laboratory sessions) on modern molecular biology and medicine. Furthermore, SMM helps students to participate in short-term scientific training in leading Polish and foreign laboratories. In parallel to funds generated by founding institutions, SMM activities were supported so far by subsidies from Polish Ministry of Health, Ministry of Science and Higher Education, Kronenberg Foundation, UNESCO-ROSTE, European Commission and National Center for Scientific Research (CNRS), France. Additional financial support came from the French government supporting the costs of participation of outstanding French scientists in tutorial and organizational activities of SMM, as well as short-term scholarships for the training of SMM students in laboratories in France.

A new international PhD program – supported by the Polish Foundation of Science providing interdisciplinary postgraduate training focusing on application of recent high throughput technologies and integrated, interdisciplinary approach to molecular genetic and genomic processes in relation to cancer development is offered by SMM.

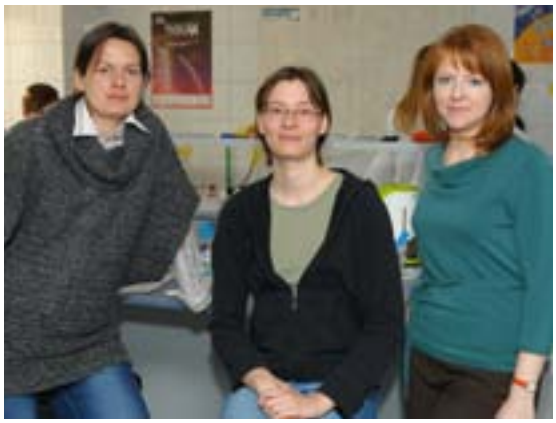
Projects are be carried out in laboratories located: in Warsaw (Warsaw Medical University, Cancer Center-Institute of Oncology, Interdisciplinary Centre for Mathematical and Computational Modelling, Nencki Institute of Experimental Biology), in Szczecin (International Hereditary Cancer Centre, Pomeranian Medical University), in Gliwice (Cancer Center-Institute of Oncology-Gliwice Branch). All teams have developed successful scientific cooperation in the proposed field of research (students will be working in collaborating laboratories for 6-12 months).

In 2009, the following courses were organized:

- SMM Spring School lecture course "From gene to phenotype – advances in molecular biology and medicine", 01-03.04.2009, Warsaw. This annual course, obligatory for first-year students, was organized by Prof. Bożena Kamińska-Kaczmarek. The lectures were given by eighteen outstanding scientists and academic teachers from the top clinical and research institutions in Poland and abroad.
- Workshop on transcriptomics, 15-17.06.2009, Warsaw, organized by SMM and Nencki Institute.
- XII Annual Inaugural and Research Report SMM Session, 29-30.10.2009, Warsaw, organized by SMM office and SMM students. Inaugural lecture was given by Prof. Patrice Debre from Université Pierre et Marie Curie, Paris.

Centre for Innovative Bioscience Education (CIBE)

(Formerly: Science Festival School)



The aim of the Centre for Innovative Bioscience Education (CIBE) is to reduce the gap between science and society in Poland by conducting educational activities which popularize biology: open lectures, workshops for students as well as courses for biology teachers. All activities are focused on improving biology education and the awareness of biology in society. Among the co-founders of the Centre for Innovative Bioscience Education there are four biological institutes, namely the International Institute of Molecular and Cell Biology (IIMCB), the Nencki Institute of Experimental Biology PAN (IBD), the Institute of Biochemistry and Biophysics PAN (IBB) and the Warsaw University of Life Sciences (SGGW), along with the BioEducation Foundation and the Warsaw Science Festival. IIMCB hosts the CIBE laboratory, office and administration. CIBE also coordinates the second laboratory at the Warsaw University of Life Sciences. In 2009, over 1,260 young participants visited laboratory workshops. At the same time, over 200 biology teachers attended laboratory workshops and courses. Over 260 children attended hands-on practical experiments.

Laboratory workshops

The participants in workshops use laboratory equipment and techniques to perform real-life experiments. The practical experiments are supported by lectures presenting the theoretical basis of molecular biology and genetics, and the techniques used in these fields of science. Every workshop lasts four hours over the course of one day. We offer the following themes:

- Explore your own DNA – examining DNA by PCR methods
- Let's play with bacteria – plasmid isolation and restriction map
- Green bacteria – bacteria transformation with GFP gene
- Protein fingerprint of different tissues
- Miracles of biotechnology – purification of jellyfish protein from bacteria
- Investigate evolution signs in your DNA – methods of molecular evolution
- Yeast – the leaving micro-factory
- Do you know what you eat?
- New! "Biotechnology of antibodies in clinical practice". Participants become acquainted with production, purification and use of different types of antibodies, including biotechnological methods allowing for large-scale manufacturing. They also perform diagnostic tests routinely used in medical practice.
- New! "Enzymes in action" During the coursework students learn basic facts regarding enzymes, such as (i) the role of enzymes in living organisms (ii) how enzymes work and (iii) what factors influence enzyme activity.

Courses for biology teachers

During our workshops for teachers we try to build a connection between them and scientists so that they can feel a part of the science community. Since we strongly encourage teachers to implement practical protocols in school curricula, we not only train them but also equip them with classroom scenarios and affordable experimental kits that can be used at school laboratories. The proposed teaching materials are an example of a state-of-art approach towards innovative bioeducation. They allow for development of practical skills and introduce a teaching approach based on projects developed by a team of students. Last but not least, our educational procedures improve ability for analytical thinking. In 2009, as part of teacher education, the following events were organized:

- Silesian Forum of Biology Teachers "Modern biology in the Polish classroom", Katowice, February 14th.
- Course for teachers "Volvox - let's teach how to experiment!", within the framework of the Science Promotion Council Meeting, Chełm, April 16th.
- Course for teachers "From an experiment to knowledge", in collaboration with the Warsaw Centre for Educational and Public Innovations and Training, Warsaw, April 28th-29th.
- Training course "Let's learn to experiment", part of a project "School biology laboratory in teachers' practice", co-financed by the Local Government of Mazowieckie Province and the BioEducation Foundation, Warsaw, June 5th-6th and October 9th-10th.
- Course for teachers from Georgia in cooperation with the Partners Foundation, Warsaw, September 22nd.
- Course for teachers "Curiosity as the key to knowledge – evolution theory inside and outside the classroom" in collaboration with the Warsaw Center for Educational and Public Innovations and Training, Warsaw, November 23rd, 25th.
- 8th CIBE and Nencki Institute Symposium for teachers

13th Science Picnic (30 May 2009)

As in previous years, the BioEducation Foundation and CIBE organized an exhibition and science show during the 13th Science Picnic in Warsaw. The 2009 motto was "Science among the stars". Our demonstrations were related to the wide use of biotechnology, spanning from visualization of DNA to biotechnology applications in the food industry.

- Can you see DNA? – isolation of DNA from onion
- Necklaces with your own DNA – isolation of DNA from the cheek
- Enzymes around us – investigation of enzyme activity
- "Tree of life ", a board game
- Food Guide Pyramid for youngsters

The XIII Science Festival (19-27 September 2009)

The Warsaw Science Festival is aimed at enhancing 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, the general public) and are run for two weeks in various universities, scientific research institutions and museums. In 2009 CIBE organized the following laboratory workshops open for the public:

- "Explore your own DNA"
- "Be like Darwin! Discover evolution!". On September 26th, CIBE organized a course entirely devoted to the Theory of Evolution. This key event for CIBE was organized because of International Darwin Year 2009. During this event participants, both children and adults, learned how fascinating the mechanisms of evolution are.
- We also hosted two lecturers from the Nencki Institute, Prof. Leszek Kuźnicki and Prof. Krzysztof Turlejski, who delivered lectures entitled "Darwinism – the past and the present" and "Evolution of the mammalian brain", respectively.

Celebration of International Darwin Year 2009

2009 was a special one for biologists due to the celebrations of the 200th anniversary of Darwin's birthday and the 150th anniversary of the publication of his seminal work "On the Origin of Species by Means of Natural Selection". CIBE organized or participated in the following events:

- Demonstration of the "Tree of life" board game during the "DNA – Life Encyclopedia" event.
- Demonstration of the "Tree of life" board game during The Science Picnic.
- Opening of the "Darwin Now" exhibition in collaboration with the British Council, the United Kingdom Embassy, and the Faculty of Biology and the Library of Warsaw University.
- Day with Darwin – the "Be like Darwin! Discover evolution!" event within the framework of the XIII Science Festival.
- Attendance at the "Communicating Darwin's Ideas: Richness and Opportunity" meeting in York (United Kingdom).
- Course for teachers "Curiosity as the key to knowledge – evolution theory inside and outside the classroom".
- Prof. Leszek Kuźnicki lecture "Darwinism – the past and the present" in the course of the VIII CIBE and Nencki Institute Symposium for Teachers.

Staff and co–workers

The administrative and coordinating staff of CIBE are: Agnieszka Chołuj, Joanna Lilpop, Marta Badurek, and Marcin Wiśniewski as a coordinator at the Warsaw University of Life Sciences.

Animators and co-workers: Krzysztof Brewczyński, Maja Cieplak, Katarzyna Chomiela, Anna Fogtman, Andrzej Foik, Damian Graczyk, Sebastian Jeleń, Kamil Koper, Marek Kulka, Maciej Kotliński, Paweł Krawczyk, Jakub Kruszewski, Aleksandra Kwiatkowska, Justyna Lesiak, Anna Łach, Kamila Ornoch, Monika Ostaszewska, Jakub Piątkowski, Malwina Pietrala, Piotr Radecki, Justyna Rudzka, Wojciech Siwek, Maciej Węsierski and Bartosz Zapisek.

Staff at IIMCB (as of 31 March 2010)

Administration		Funding
Jacek Kuźnicki	Director	IIMCB
Michał Witt	Deputy Scientific Director	IIMCB (1/2)
Hanna Iwaniukowicz	Financial Manager	IIMCB
Agnieszka Karbowska	Director's Representative for Administrative Matters	IIMCB
Agnieszka Wagner-Ziemka	Director's Representative for Research Management	IIMCB/EU grant
Roman Szczepanowski	Director's Representative for Information Technology & Research Equipment	EU grant (1/2)
Dominika Dubicka	Director's Assistant	IIMCB
Monika Nowicka	Payroll Specialist	IIMCB
Renata Knyziak	Accounting Specialist	IIMCB/Polish-Norwegian Res. Fund
Mariola Arkuszewska	Accounting Specialist	IIMCB/Structural Funds
Krystyna Domańska	Human Resources Specialist	IIMCB (1/2)
Beata Tkacz	Human Resources Specialist	IIMCB
Urszula Białek-Wyrzykowska	International Cooperation Manager	IIMCB (1/2)
Dorota Wasiak-Libiszowska	Foreign Grants Manager	IIMCB/Structural Funds
Magdalena Powierża	International Cooperation Specialist	IIMCB/EU grant
Marcin Ogonowski	International Cooperation Specialist	IIMCB/Structural Funds
Anna Brzezińska	Tender Specialist	IIMCB
Dorota Makulska	Secretary	IIMCB
Robert Banasiak	Maintenance Specialist	IIMCB

Department of Molecular Biology		
Maciej Żylicz	Head	IIMCB
Alicja Żylicz	Vice Head	IIMCB
Marcin Klejman	Research Associate	IIMCB
Dawid Walerych	Research Associate	IIMCB (1/2) Ministerial grant (1/2)
Paweł Wiśniewski	Research Associate	EU Grant
Marta Małuszek	Junior Researcher	IBB PhD School/Ministerial grant
Zuzanna Szymańska	Junior Researcher	ICM
Zuzanna Tracz	Junior Researcher	IBB PhD School/Ministerial grant
Jakub Urbański	Junior Researcher	Utrecht University Fellowship
Milena Wiech	Junior Researcher	Nencki PhD School/Ministerial grant
Natalia Sikorska	MSc student	Volunteer
Aleksandra Dudek	MSc student	Volunteer
Grażyna Orleańska	Secretary	IIMCB (1/2)

Laboratory of Structural Biology MPG/PAN		
Matthias Bochtler	Head	Max Planck Society
Honorata Czapińska	Post doctoral Fellow	EU grant
Renata Filipek	Post doctoral Fellow	EU grant
Izabela Sabała	Post doctoral Fellow	EU grant
Monika Sokołowska	Post doctoral Fellow	Max Planck Society/EU grant
Roman Szczepanowski	Post doctoral Fellow	Ministerial grant (1/2)
Grzegorz Chojnowski	Junior Researcher	Ministerial funds*
Patrycja Haniewicz	Junior Researcher	EU grant/Nencki PhD School
Henryk Korza	Junior Researcher	EU grant
Marek Wojciechowski	Junior Researcher	IIMCB/Nencki PhD School
Jean-Philippe Borges	EU visiting expert	EU grant

Laboratory of Biomodelling		
Sławomir Filipek	Head	IIMCB
Michał Koliński	Senior Researcher	EU grant
Dorota Latek	Senior Researcher	Structural Funds
Szymon Niewieczerzał	Senior Researcher	Structural Funds
Bartosz Trzaskowski	Senior Researcher	FNP Homing
Aleksander Dębiński	Junior Researcher	Warsaw University Fellowship
Wojciech Puławski	Junior Researcher	IIMCB/IBB
Krzysztof Młynarczyk	Junior Researcher	Warsaw University Fellowship
Umesh Ghoshdastider	Junior Researcher	EU grant

* – Ministerial matching funds to EU grant

Laboratory of Bioinformatics and Protein Engineering		
Janusz M. Bujnicki	Head	IIMCB
Krzysztof Skowronek	Research Coordinator	IIMCB
Michał Boniecki	Post-doctoral Fellow	Ministerial grant
Małgorzata Durawa	Junior Researcher	EU grant
Agata Kamaszewska	Junior Researcher	Ministerial grant
Katarzyna H. Kamińska	Junior Researcher	Ministerial funds*
Iga Korneta	Junior Researcher	EU grant
Łukasz Kozłowski	Junior Researcher	Ministerial funds*
Jerzy Orłowski	Junior Researcher	IIMCB
Marcin Pawłowski	Post-doctoral Fellow	EU grant
Dariusz Pianka	Junior Researcher	Ministerial funds*
Michał Piętal	Junior Researcher	NIH grant
Katarzyna Poleszak	Junior Researcher	IIMCB
Wojciech Potrzebowski	Junior Researcher	Ministerial grant
Elżbieta Purta	Specialist	EU grant
Wojciech Siwek	Junior Researcher	Ministerial funds*
Irina Truszyńska	Junior Researcher	Ministerial grant
Ewa Wywiał	Programmer	BIOCENTRUM
Maria Werner	Junior Researcher	Ministerial grant
Paweł Łukasz	MSc Student	NIH grant
Magda Bogdał	MSc Student	Volunteer
Albert Bogdanowicz	MSc Student	Volunteer
Natalia Borkowska	MSc Student	Volunteer
Anna Górska	MSc Student	Volunteer
Małgorzata Habich	MSc Student	Volunteer
Piotr Iwaniuk	MSc Student	Volunteer
Jakub Jopek	MSc Student	Volunteer
Magdalena Mika	MSc Student	Volunteer
Sebastian Opałczyński	MSc Student	Volunteer
Tomasz Stępniewski	MSc Student	Volunteer
Krzysztof Suchoński	MSc Student	Volunteer
Piotr Wojciechowski	MSc Student	Volunteer
Katarzyna Ziółkowska	MSc Student	Volunteer
Agnieszka Faliszewska	Office Manager	EURASNET
Jan Kogut	Computer Administrator/Programmer	BIOCENTRUM
Tomasz Jarzynka	Computer Administrator/Programmer	BIOCENTRUM
Łukasz Munio	Computer Administrator	Ministerial grant

Laboratory of Neurodegeneration		
Jacek Kuźnicki	Head	IIMCB
Urszula Wojda	Associate Professor	IIMCB
Joanna Gruszczyńska-Biegała	Post-doctoral Fellow	Ministerial grant
Tomasz Węgierski	Post-doctoral Fellow	EU grant
Marta Wiśniewska	Post-doctoral Fellow	EU grant
Emilia Białopiotrowicz	Junior researcher	Ministerial grant/Nencki PhD School
Katarzyna Dębowska	Junior researcher	Ministerial grant/Nencki PhD School
Katarzyna Misztal	Junior researcher	IIMCB/Nencki PhD School
Andrzej Nagalski	Junior researcher	IIMCB/Nencki PhD School
Aleksandra Szybińska	Junior researcher	IIMCB
Mateusz Ambrożkiewicz	MSc Student	Volunteer

Laboratory of Molecular and Cell Neurobiology		
Jacek Jaworski	Head	IIMCB/Polish-Norwegian Res. Found
Magda Błażejczyk	Post-doctoral Fellow	Polish-Norwegian Research Found
Iwona Cymerman	Post-doctoral Fellow	EU grant
Agata Góźdź	Post-doctoral Fellow	EU grant
Matylda Macias	Post-doctoral Fellow	EU grant/Nencki Institute
Anna Malik	Junior researcher	Nencki PhD School
Małgorzata Perycz	Junior researcher	Ministerial grant/Nencki PhD School
Łukasz Świech	Junior researcher	Nencki PhD School
Agnieszka Skałecka	Junior researcher	Era-Net Neuron grant
Malgorzata Urbańska	Junior researcher	Ministerial funds*/Nencki PhD School
Anna Urbańska	Junior researcher	Ministerial grant/Nencki PhD School
Paweł Krawczyk	MSc Student	Volunteer
Kamil Parobczak	MSc Student	Volunteer
Patrycja Pietruszka	MSc Student	Volunteer
Małgorzata Zarębska	MSc Student	Volunteer

* – Ministerial matching funds to EU grant

Laboratory of Cell Biology		
Marta Miączyńska	Head	Wellcome Trust
Iwona Pilecka	Post-doctoral Fellow	Wellcome Trust
Beata Pyrzyńska	Post-doctoral Fellow	HHMI
Ewelina Szymańska	Post-doctoral Fellow	EU grant
Maciej Lipko (joint with Department of Molecular Biology)	Post-doctoral Fellow	Polish-Norwegian Research Fund
Magdalena Banach-Orłowska	Research Assistant	Wellcome Trust
Anna Hupałowska	Junior Researcher	EU grant/Nencki PhD School
Marta Olchowik	Junior Researcher	HHMI/Nencki PhD School
Agnieszka Pawlik	Junior Researcher	IIMCB/Nencki PhD School
Łukasz Sadowski	Junior Researcher	EU grant/Nencki PhD School
Anna Toruń	Junior Researcher	IIMCB/Nencki PhD School
Anna Urbańska	Junior Researcher	Ministerial grant/Nencki PhD School
Izabela Sępowicz	Grant Administrator and Lab Manager	Polish-Norwegian Res. Fund/ Structural Funds

Laboratory of Cell Cortex Mechanics MPG/ PAN		
Ewa Paluch	Head	IIMCB
Jakub Sędzinski	Junior Researcher	Ministerial grant
Maté Biro	Junior Researcher	Ministerial grant
Alba Diz Muñoz	Junior Researcher	DFG Grant
Andrew G. Clark	Junior Researcher	Ministerial grant
Martin Bergert	Junior Researcher	DFG Grant
Steve Simmert	MSc Student	Volunteer
Martine Ruer	MSc Student	Volunteer
Stanley Dinesh Chandradoss	MSc Student	Volunteer
Julia Roensch	Technician	Ministerial grant

Laboratory of Protein Structure		
Marcin Nowotny	Head	Wellcome Trust
Elżbieta Nowak	Post-doctoral Fellow	EU grant
Karolina Górecka	Post-doctoral Fellow	Wellcome Trust
Małgorzata Figiel	Junior Researcher	IIMCB
Marcin Jaciuk	Junior Researcher	IIMCB
Jakub Jurkowski	Junior Researcher	Ministerial grant
Mirosław Śmietański (joint with Lab. of Bioinf. & Prot. Eng)	Junior Researcher	Ministerial funds*
Monika Rychlik	Lab Manager	Wellcome Trust

Laboratory of Mitochondrial Biogenesis		
Agnieszka Chacińska	Head	IIMCB/ FNP Welcome
Piotr Brągoszewski	Post-doctoral Fellow	IIMCB/FNP Welcome
Magdalena Kaus-Drobek	Post-doctoral Fellow	FNP Welcome/Ministerial grant
Adrianna Łoniewska-Lwowska	Post-doctoral Fellow	IIMCB
Tomasz Czerwik	PhD Student	FNP Welcome/IBB PhD School
Agnieszka Górnicka	PhD Student	FNP Welcome/IBB PhD School
Aksana Varabyova	PhD Student	Ministerial grant/Nencki PhD School
Lidia Wróbel	PhD Student	FNP Welcome/Nencki PhD School
Anita Chodkowska	Research Assistant	FNP Welcome
Paulina Kwiatkowska	Undergraduate Student	Volunteer
Inmaculada Mora Espi	Undergraduate Student	Volunteer/FNP Welcome
Kamila Ornoch	Undergraduate Student	Volunteer/FNP Welcome

PolSenior Project		
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Aleksandra Szybalska	Project Assistant	Ministerial grant
Magdalena Owczarz	Assistant	Ministerial grant
Przemysław Ślusarczyk	IT Specialist	Ministerial grant
Marta Świech	Technician	Ministerial grant
Ewa Tondys	Technician	Ministerial grant
Malwina Wawrzyniak	Technician	Ministerial grant

Research Equipment Laboratory		
Wanda Gocal	Technician	IIMCB
Monika Dudek	Technician	IIMCB
Jadwiga Dyttus	Technician	IIMCB
Elżbieta Grzelak	Technician	IIMCB

* – Ministerial matching funds to EU grant

Centre for Innovative Bioscience Education		
Agnieszka Chołuj	Head	IIMCB/Nencki/IBB
Joanna Lilpop	Coordinator	Nencki/IBB
Marta Badurek	Coordinator	Nencki/IBB
Marcin Wiśniewski	Coordinator	SGGW
Anna Fogtman	Teacher	Volunteer
Justyna Rudzka	Teacher	Volunteer
Kamil Koper	Teacher	Volunteer
Aleksandra Kwiatkowska	Teacher	Volunteer
Maciej Kotliński	Teacher	Volunteer
Maja Cieplak	Teacher	Volunteer
Kamila Ornoch	Teacher	Volunteer
Andrzej Foik	Teacher	Volunteer
Piotr Radecki	Teacher	Volunteer
Katarzyna Chomiela	Teacher	Volunteer
Marek Kulka	Teacher	Volunteer
Krzysztof Brewczyński	Teacher	Volunteer
Jakub Kruszewski	Teacher	Volunteer
Monika Ostaszewska	Teacher	Volunteer
Anna Łach	Teacher	Volunteer
Justyna Lesiak	Teacher	Volunteer
Michał Mlącki	Teacher	Volunteer
Malwina Pietrala	Teacher	Volunteer

Health Prot Proteins in Health and Disease

WP1 - Increasing scientific expertise through twinning

1. **Matthias Bochtler**, Laboratory of Structural Biology, IIMCB and **Ruedi Allemann**, University of Cardiff, UK
2. **Janusz M. Bujnicki**, Laboratory of Bioinformatics and Protein Engineering, IIMCB and **Saulius Klimasauskas**, Laboratory of Biological DNA Modification, Institute of Biotechnology, Vilnius, Lithuania
3. **Sławomir Filipek**, Biomodelling Laboratory, IIMCB and **Vicenza Andrisano**, Department of Pharmaceutical Sciences, University of Bologna, Italy
4. **Jacek Jaworski**, Laboratory of Molecular and Cellular Neurobiology, IIMCB and **Casper Hoogenraad**, Erasmus MC, Rotterdam, The Netherlands
5. **Jacek Kuźnicki**, Laboratory of Neurodegeneration, IIMCB and **Jochen Herms**, Ludwig-Maximilians-University of Munich, Centre for Neuropathology, Germany
6. **Marta Miączyńska**, Laboratory of Cell Biology, IIMCB and **Harald Stenmark**, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway
7. **Marcin Nowotny**, Laboratory of Protein Structure, IIMCB and **Roland Marquet**, Retroviruses and RNA viruses laboratory, RNA Architecture and Reactivity Unit, Université Louis Pasteur, CNRS, Strasbourg, France
8. **Michal Witt's** group, Ciliary Proteins Function Project, IIMCB and **Heimut Omran**, Department of Pediatrics and Adolescent Medicine, University of Freiburg, Germany
9. **Maciej Żylicz**, Department of Molecular Biology, IIMCB and **Ted Hupp**, Cancer Research of UK Cell Signalling Unit, Edinburgh Cancer Research Centre, University of Edinburgh, UK

WP2 - Expanding research capacity

Employment of **9 experienced scientists** for 2 years each and an Equipment Specialist.

WP3 - Organization of scientific events

Workshops, courses, seminars related to: ciliary disorders, cancer biology, DNA repair, neurobiology and neurodegenerative disorders, finding pathways between proteins, biology of antibiotic resistance, heat shock proteins in molecular medicine, cell biology of endocytosis. Most of the topics are within special interest of EC: rare disorders, cancer, neurodegenerative disorders, HIV.

WP4 - Participation in international events

Participation of Centre's staff in international conferences and courses

WP5 - Promotion

Project's website, annual reports on HEALTH-PROT activities, leaflets, posters, organization of public events, open days.

WP6 - Management

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