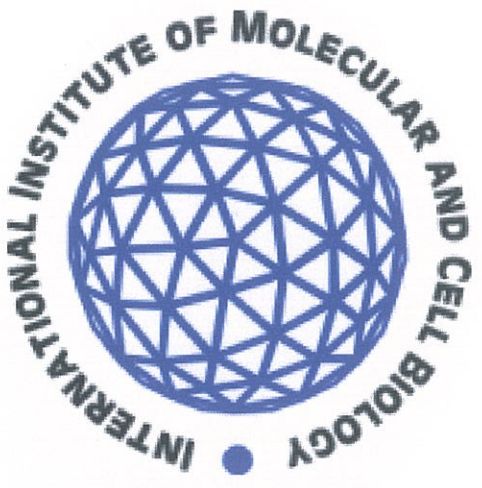

International Institute of Molecular and Cell Biology in Warsaw

Annual Report 2000

Contents

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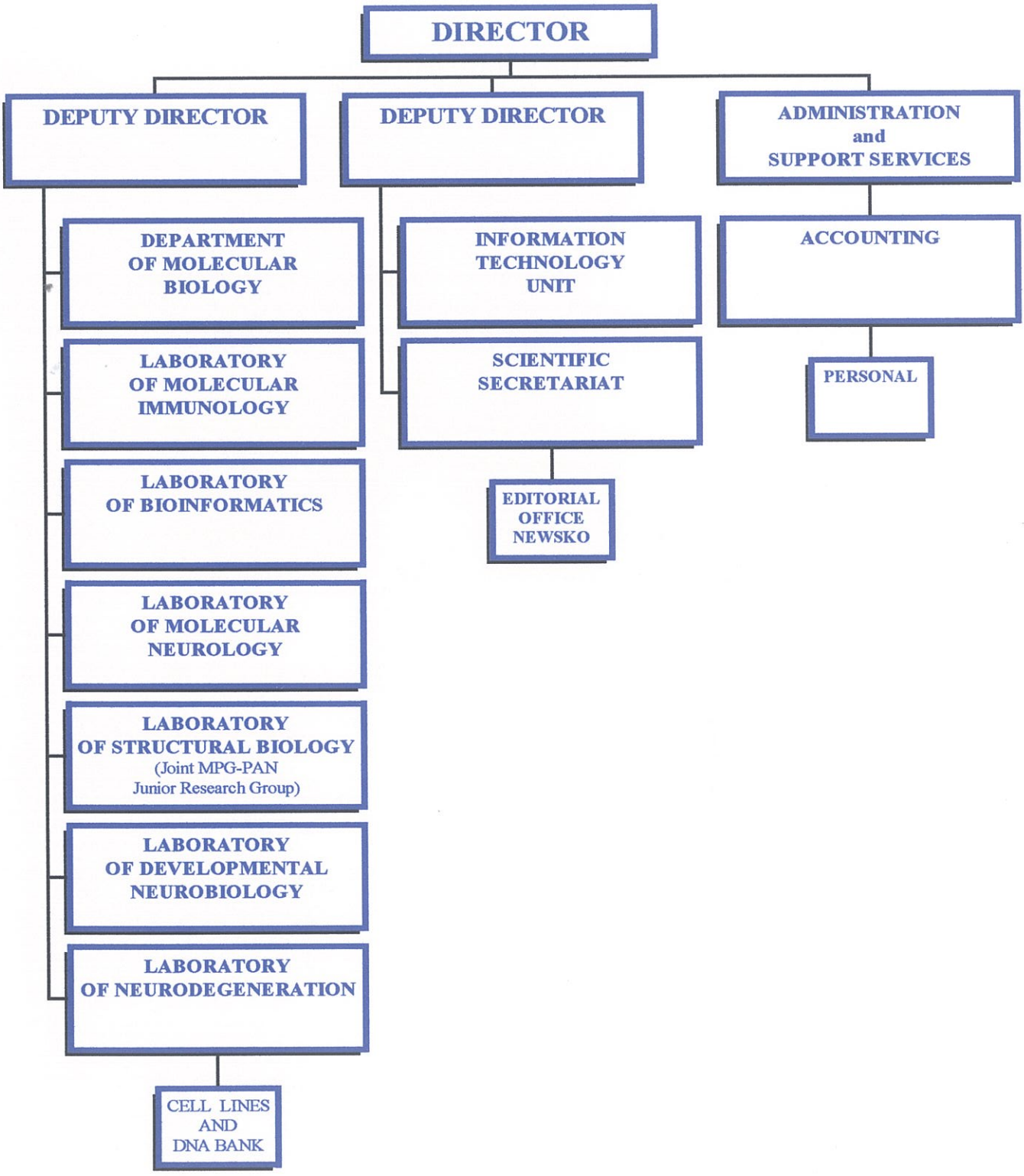
Structure of the Institute

(as of May 10th, 2001)



International Advisory Board

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Ryszard Przewłocki, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna St., 31-343 Cracow, Poland

Sir Marc Richmond, School of Public Policy, University College London Brookhouse 2-16, Tarrington Place, London WC1E 7HN, Great Britain

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Michal Witt*, Deputy Director, International Institute of Molecular and Cell Biology in Warsaw; Institute of Human Genetics, Polish Academy of Sciences, 32 Strzeszynska St., 60-479 Poznan, Poland

Maciej Zylicz*, Head, Department of Molecular Biology, International Institute of Molecular and Cell Biology in Warsaw, 4 Trojdena St., 02-109 Warsaw, Poland; Head, Biology, Earth Sciences and Environmental Protection Commission, State Committee for Scientific Research and member of the State Committee for Scientific Research

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* These persons have resigned from the participation in the IAB activities due to their positions as deputy directors (JK and MW), and head of the Department (MZ).

Directors' Report

Introduction

Towards a new international biological institute in Warsaw (Excerpts from: "Net News", Newsletter of the UNESCO Global Network for Molecular and Cell Biology, 1 October 1991). Maciej J. Nalecz and Leszek Kuznicki wrote:

Present time witness an astonishing progress in biomedical sciences. Initiatives like "Human Genome Project" and "Decade of the Brain", anti-AIDS and anti-tumor research, fights against the Alzheimer's disease, advanced studies of the cell cycle mechanisms, of action of hormones and vitamins or of general metabolic regulatory pathways, now running and supported by most developed countries, are all directed to improve human life. Also, they are all parts of the field that may be generally called "cell and molecular biology". This field promises to develop further, well into the next century, and to remain for a number of years very productive. Thus countries now under way of changing, willing to catch-up with the world and to develop accordingly, are bound to promote studies on cell and molecular biology to the respective level. It will be a long and systematic process, but initiation can be much faster. The first step could occur in Poland whose tradition and the present status seems especially suitable, as discussed above. What we would like to propose is to organize in Warsaw an International Institute of Cell and Molecular Biology. A modern institute as planned, in the field of molecular and cell biology, is bound to have a remarkable fallout in a number of domains, not only strictly related to science but, even more important, related to the general productivity and development of societies. It is expected that the establishment of the institute will provide conditions for fast, coordinated and well organized development of cell and molecular biology research and application in Poland, and through it, also in other countries of the East European region. It will stimulate formation of modern pharmacological, biotechnological industry in the region, as well as provide a center of excellence for training of Ph.D. and post-doctoral students, of medical students and graduates in the fields of cell and molecular biology. Actually, it is presumed that the institute will run a special training programme, unique in the region, thus giving a profound cultural impact on East European science and promoting integration of biologists, pharmacologists and clinicians interested in using modern methods of cell and molecular biology. It is also expected that a center of excellence formed by the institute will attract a number of emigrated and now well-trained and established researchers of Polish origin from leading Western laboratories. We know that some of them are already enthusiastic about the idea and ready to help, and many others will surely follow.

Important dates in the Institute's history

September	'91,	Proposal to create the Institute was published in the UNESCO Bulletin of MCBN
June	'94,	KBN accepts the activities aimed at establishing the Institute
October	'94,	Presidium of PAN votes to support the Institute
May	'95,	Agreement between Poland and UNESCO to establish the Institute
June	'96,	Molecular and Cell Biology Department is created by PAN
June	'97,	Parliament passes the bill to found the Institute
December	'98,	Department of Molecular and Cell Biology is dissolved
January	'99,	Institute starts its independent activities
July	'99,	Dr. Dastych appointed as a leader of the Laboratory of Molecular Immunology
October	'99,	Dr. Zylicz appointed as a Chairman of the Department of Molecular Biology
April	'00,	Agreement between Max Planck Society (MPG) and Polish Academy of Sciences (PAN) on launching the Joint MPG-PAN Junior Research Group
November	'00,	Dr. Bochtler appointed as a leader of the Laboratory of Structural Biology (Joint MPG-PAN Junior Research Group), and Dr. Hetman appointed as a leader of the Laboratory of Molecular Neurology
December	'00,	Dr. Rychlewski appointed as a leader of the Laboratory of Bioinformatics

Descriptions of the Institute's activities in 2000

Organization of research at the Institute

Research at the Institute is being conducted in six major areas defined by interest of principal investigators. Dr. Dastych studies the molecular mechanisms of immunotoxicity: mast cell mediated modulation of immune response by immunotoxic agents and search for molecular targets of immunotoxic compounds. Prof. Zylicz projects include: analysis of interactions between human p53 and cytosolic molecular chaperones, Hsp as a vehicle for protein therapy, cloning and characterization of a novel human protein kinase and a search for new Hsp homologues. Prof. Kuznicki is interested in molecular events leading to a genetically linked form of Alzheimer disease (FAD) and in genetic aspects of longevity. Dr. Rychlewski works on the improvement of the research prediction methods and on the broader application of the developed tools and the promotion of utilization of the achievements of bioinformatics in biomedical research. Dr. Hetman is at the stage of initiation of his projects on molecular basis of neuronal apoptosis. Dr. Bochtler is currently organizing his structural biology laboratory, funded by Max Planck Society (*see section "International contacts", p. 12*), to start the project on structural analysis of ubiquitin activating enzymes, ligases and hydrolases, in selected protein conjugation systems. Dr. Matter (employment starting September 2001) will be working on transcriptional codes specifying neuronal identity during neurogenesis in vertebrates.

Education has been carried out through our own Ph.D. programme (5 students), in collaboration with the Utrecht University, The Netherlands (3 students: two in Warsaw, one in Cracow), with the Postgraduate School of Molecular Medicine (1 student), with the Kosciuszko Foundation (1 student from USA) *(see section "Educational activities", Ph.D. Programme, p. 37),* and by organization of scientific meetings and courses (3 Ph.D. and 4 M.Sc.).

Implementation of research results. Basic research is the core activity of the Institute, however, the possible applications of our research are always being considered and, if possible, developed (e.g. participation of the Institute's staff involved in the Centenarians and Alzheimer Disease Projects in the PHARE Center of Excellence coordinated by the Nencki Institute). In addition to that, some projects are directly dedicated to applied research, such as those developed by Dr. Rychlewski and Dr. Zylicz.

Popularization of science has been achieved through participation in the Science Festival session on mechanism of longevity (Warsaw, September 21, 2000) and by participation of faculty members in TV and radio programmes, by writing articles and giving interviews in newspapers.

Editing NEWSKO, an electronic bulletin on scientific events on the Ochota Campus (http:/www.iimcb.gov.pl/newsko/newsko.htm). The first issue of this weekly bulletin was sent out to readers on September 10th, 1998. Since then, the bulletin reaches its readers every Thursday with information on lectures and seminars on the Campus in the following week, as well as about the jobs available and questions from and to the scientific community of Ochota Campus. At present, there are more than 200 subscribers, and the number is growing. At the end of December 2000 the 93 issue was sent out.

Directors' note

As for mid-May, 2001, it can be proudly announced that the initial stage of the Institute's organization has been completed. Since the last Report significant changes at the Institute's structure and activity took place. First, six groups have initiated their research, five in "wet" labs and one in bioinformatics lab. One of the laboratories is sponsored by the Max Planck Society and this adds a lot of prestige to the Institute. A new lab chief appointment has been made for a seventh laboratory, and it will start its activity in September. Second, more than half of the Institute's space has been filled with the laboratory furniture and equipment, and more than 40 people work there every day. Third, the financial situation has improved and the funding of the Institute is now based on a variety of different Polish and foreign sources including grants from European Union. Last but not least, first papers have already been published based on the work conducted at the Institute with its name and address as the official affiliation of the authors. In summary, we believe that the critical mass of the Institute has been reached, and that it has now became already an established scientific entity, recognized in Poland and abroad.

Angelo Azzi, Jacek Kuznicki, Michal Witt

Grants, donations in 2000

International

- **UNESCO** support for the preparatory activity to establish a new UNESCO Professorship Chair at the IIMCB; 20,000 USD

- **UNESCO** support for the IIMCB; 30,000 USD annually in 2000 and 2001, received 15,000 USD, (M. Zylicz)

- **UNESCO** support for the Polish Centenarians Project; 10,000 USD, (J. Kuznicki)

- **5th Framework Programme** + **KBN** "A New Technology for Fluorescent "Cell Chip" Immunotoxicity Testing"; 231,703 EUR (EU) + 207,000 EUR (KBN), 2001-2003, (J. Dastych)

- **5th Framework Programme** + **KBN** "Rational engineering of lipid metabolism in flax"; 149,000 EUR (EU) + 135,000 EUR (KBN), 2001-2003, (L. Rychlewski)

- **Max-Planck Society (MPG) - Polish Academy of Sciences (PAN) Junior Research Group Programme**; 1,300,000 DM for equipment and 240,000 DM annually for 5 years, (M. Bochtler)

- Participation in the Nencki Institute Center of Excellence entitled "Center of studies on mechanisms of neurodegeneration and methods to prevent it", within the **PHARE SCI-TECH II PL** Project; 4,000 EUR for IIMCB, (J. Kuznicki)

- **R.W. Johnson Institute and UCSD**, "FFAS server and structure prediction database"; 45,000 USD, 2000, (L. Rychlewski)

- **Utrecht University** fellowships for three Ph.D. students (in M. Zylicz and J. Dastych labs at IIMCB, and one in Cracow in R. Przewlocki lab); 55,000 Hfl annually from 2000 to 2003

- 10 months fellowship for Ms. Sanne Mikkelsen, M.Sc. from the **Polish Academy of Sciences** and the **Danish Academy of Sciences** to perform studies at J. Kuznicki's laboratory at IIMCB

- **Private donation** from Krystyna and Bolesław Wydzga, L'anza Research International, USA for the Polish Centenarians Project; 15,000 PLN, (J. Kuznicki)

Polish

- Grant from the State Committee for Scientific Research (KBN 6P04A04219) "Interaction of chaperones with p53 protein"; 447,000 PLN for 3 years (2000-2002), (M. Zylicz)

- Grant from Foundation for Polish Science (FNP 15/2000) "Molecular chaperones in cancer cells"; 225,000 PLN for 3 years (2000-2002), (M. Zylicz)

- Equipment Grant from State Committee for Scientific Research (KBN); 500,000 PLN for Molecular Medicine Research Group based on agreement signed by IIMCB, Nencki and IBB Institutes

- Grant from the State Committee for Scientific Research (KBN) "Molecular characteristics of immunomodulatory mechanism of Hsp70 complex and endogenous peptides"; approx. 40,000 PLN for equipment, (coordinator G. Galazka, cooperators: A. Selmaj and A. Wawrzynow)

- Grant from the State Committee for Scientific Research (KBN 1114/P04/2000/19) "Cloning and characterization of a novel human protein kinase"; 386 500 PLN for 3 years (2000-2003), (L. Trzeciak)

- Research Grant from the State Committee for Scientific Research (KBN 4P05A05118) entitled "Characterization of promoter sequences, transcription factors and signal transduction pathways involved in activation of IL-4 gene expression by heavy metal ions"; 180,000 PLN (2000-2003), (J. Dastych)

- Research Grant from the State Committee for Scientific Research (KBN 6P04A03919) for young investigator, entitled "Influence of 3'UTR sequences of IL-4 gene on stability of mRNA"; 18,200 PLN (2000-2003), (D. Trzaska)

- Grant from the State Committee for Scientific Research (KBN) support of 5th FP application "European Structure Prediction Network"; 15,000 PLN, (L. Rychlewski)

- Grant from the State Committee for Scientific Research (KBN) "Engineering of DNA methytransferases"; 200,000 PLN, (2000-2002), (coordinator - M. Radlinska, cooperators: L. Rychlewski, J. Bujnicki), coordinated at the Biology Department of UW)

Organization of conferences:

Spring School *"Genetics on the Turn of Centuries"*, Postgraduate School of Molecular Medicine (SMM), Poznan, May 15-16, 2000 (organizer: M. Witt)

EFIS 2000 Satellite Symposium. *"Heat Shock Proteins: Immune, Stress Response and Apoptosis"*, Gdansk, September 21-23, 2000 (organizer: M. Zylicz)

Session during XXXVI Polish Biochemistry Society Conference *"DNA Replication and Repair"*, Poznan, September 11-15, 2000 (organizer: M. Zylicz)

MCBN/UNESCO-Max Planck Society-Polish Academy of Sciences Symposium and Workshop *"Impact of Modern Biology on Society"*, Warsaw, October 4-5, 2000 (organizer: M.J. Nalecz)

Conference of the Polish Working Group for Cystic Fibrosis, Warsaw, November 24, 2000 (organizer: M. Witt)

Inauguration and review session of students of the Postgraduate School of Molecular Medicine (SMM), Warsaw, November 27-28, 2000 (organizer: L. Kaczmarek)

IV International Symposium and Integrated Course on Molecular Medicine (SMM), *"Molecular Basis of Hypertension"*, Warsaw, November 29 - December 1, 2000 (organizer: Z. Gaciong)

CAFASP-2 Meeting *"Critical Assessment of Fully Automated Structure Prediction"* http://www.cs.bgu.ac.il/~dfischer/CAFASP2/) conducted as a part of the CASP4 Meeting *"Critical Assessment of Structure Prediction"*, Asilomar Conference Center, California USA, December 3 - 7, 2000 (coorganizer L. Rychlewski)

Participation in organization of the 2nd International Conference on Protein Kinases Inhibitors, Warsaw, 2001 (J. Kuznicki, M. Mossakowska)

International contacts

With Max Planck Society

As a result of several visits of highly ranked officials of the Max Planck Society including Prof. Halbrock, on April 3rd, 2000 a letter of intent between the Max Planck Society and the Polish Academy of Sciences was signed by Prof. M. Mossakowski, President of PAN and Prof. H. Markl, President of MPG. The letter allowed to initiate a Joint MPG-PAN Junior Research Group Programme (JJRGP) located at the IIMCB. The aims of the programme are: (1) promotion of innovative research topics of common interest in which partner organizations have outstanding capabilities and which complement existing activities and expertise, (2) help with the implementation of a novel mechanism of selection and promotion of young and talented researchers in the institutes of PAN which has already proven to be efficient and successful in the institutes of MPG, (3) strengthening of scientific cooperation between MPG and PAN. Within an international competition for the JJRGP position, 7 candidates (out of 22 applications) were audited during the symposium held on October 4th, 2000 at IIMCB. As a result Dr. Matthias Bochtler from Martinsried, Germany was selected as a leader of the group funded by MPG and organized at IIMCB. Dr. Bochtler is officially employed at the Max-Planck Institute of Molecular Cell Biology and Genetics in Dresden since November 15th, 2000 and his 5-year contract allows him to conduct the research in the field of crystallography at IIMCB in Warsaw.

With Utrecht University

This is a part of the research collaboration programme initiated by Prof. Willem Gispen to facilitate the exchange of scientific information and ideas among Polish and Dutch scientists and graduate students and allow for short-time research visits of the staff members and their students from Poland to Utrecht and vice versa. For more details see section *Educational activity*, p. 37.

With UNESCO

MCBN/UNESCO – Max Planck Society – Polish Academy of Sciences organized Symposium and Workshop on *"Impact of Modern Biology on Society"*, October 4-5, Warsaw. The programme has been composed of two complementary subsequent events – Symposium (with lectures highlighting recent discoveries in cell and molecular biology, first day) and Workshop (relating these discoveries to needs and concerns of the modern society, second day).

Participants: There were 127 registered participants, but some additional listeners (mainly students of the Warsaw University Campus) were allowed to take part in both events. Also, several journalists joined the Workshop and participated in the press conference organized at the end of the meeting.

Among the lecturers were: A. Azzi (MCBN/UNESCO), M. Bochtler (MPG), A. Dulak (Vienna University), I. Gates (MPG), M. Gierdalski (PAN), W.H. Gispen (Utrecht University), K. Hahlbrock (MPG), W. Huttner (MPG), A. Jerzmanowski (Warsaw University), L. Kaczmarek (PAN), B. Kaminska (PAN), J. Kuznicki (PAN), A. Legocki (PAN), J. Mozrzymas (ICGEB Trieste & Wroclaw University), M. Nalecz (PAN), N. Nelson (Tel-Aviv University), W. Patzel (Hamburg University), L. Rychlewski (PAN), K. Simons (MPG), W. Stec (PAN), M. Witt (PAN) and M. Zylicz (PAN & Gdansk University). Additional participants were invited for the second day (Workshop), to present short statements and to actively participate in the round-table discussion. Those included: Prof. Magdalena Fikus (Chairman of the Biochemistry and Biophysics Committee of PAN), Prof. Aleksander Koj (former Rector of the Jagiellonian University, member of the Bioethics Committee of PAN), Prof. Leszek Kuznicki (former President of PAN, Chairman of the Polish ICSU Commission), Prof. Miroslaw Mossakowski (President of PAN), Prof. Witold Karczewski (former Chairman of the Polish State Committee for Scientific Research, President of the Polish State Commission on Ethics in Research) and Prof. David Shugar (foreign Member of PAN, expert on bioethics).

The first day (Symposium) has been chaired by Prof. Klaus Hahlbrock (Deputy President of the Max Planck Society for Life Sciences) and Prof. Andrzej Legocki (Co-Director of the Polish MCBN/UNESCO Network). The second day (Workshop) has been moderated by Prof. Angelo Azzi (Chairman of MCBN/UNESCO) and Prof. Maciej Nalecz (Co-Director of the Polish MCBN/UNESCO Network).

The Symposium covered following subjects: molecular mechanisms of action of environmental pathogens, neurodegenerative diseases – mechanisms and social implications, biological basis for longevity, new molecular biology developments in clinical application – profits and dangers, prions and related pathologies, traditional medicine *versus* modern pharmacology, and bioinformatics – science of the XXIst century.

The Workshop concentrated on the following:

Information and society. Prof. Azzi quoted the statement made by Bruce Alberts, President of the National Academy of Sciences (USA). "The links between science and society will become tighter and more numerous in the coming century". He outlined "an increasing role for global citizen scientists" as "our social institutions have an increasing need for individuals who can stand at the interface between new knowledge, on the one hand, and major national and international societal needs". This, he said, is due to two social trends, "the advent of a global information-based economy and [...] the growing internationalization of science itself". Along these lines the aspects of a developing science and a developing society (as well in different cultures) in terms of compatibility between accepted facts and scientific challenges have been discussed.

The responsibility of scientists. Sir Joseph Rotblatt said that "The general public, through elected governments, has the means to control science; either by withholding the purse, or by imposing restrictive regulations harmful to science: Clearly, "he recommended, "it is far better that any control should be exercised by the scientists themselves." He called on scientists "to show by their conduct that it is possible to combine creativeness with compassion" and recommended that science students, before graduation, be made to sign a code of conduct comparable to the Hippocratic Oath which has been in existence for physicians in the West for some 2,500 years. Sir Joseph Rotblatt further recommended the establishment of independent ethical committees of scientists to oversee research projects, and denounced the patenting of scientific findings as going "against a basic tenet of science". Finally, he called for an end to scientists' involvement in arms research projects. "The basic human value is life itself", he declared, "the most basic of human rights is the right to live". Following these postulates the discussion has been carried out on the responsibility that scientists have for their own discoveries and the need that a control of discoveries be made by scientists, in the interest of a better employment of science.

The problem of cloning. The new-found capability to clone a "Dolly" carries a more complex societal context than discovering the double helix design of DNA, and thus demands extensive public discussion

and debate. The ability to do this new cloning requires us to examine and discuss our society's philosophical and religious underpinnings, its legal definitions and ethical values - all in relation to the application of this revolutionary new skill. "I believe (quotation from Dr. Neal Lane, Director, National Science Foundation, USA) that it also suggests to individuals the need to examine their own personal values and beliefs in light of the possibilities." Along these lines a discussion developed on the values of cloning animal species, in terms of obtaining useful information for mankind. The limit has been recognized to be set at the level of cloning of a human being. On the other side the usage of stem cells of human origin has been recommended as a valid tool to understand and cure diseases.

In fact, our success in managing knowledge and technology to solve a vast array of social problems is directly dependent on this very social context of values and ethics.

Department of Molecular Biology

Staff:
Dr. Maciej Zylicz (Head), Dr. Alicja Wawrzynow (Associate Professor), Dr. Grazyna Mosieniak, Dr. med. Lech Trzeciak, Dr. Agnieszka Wawrzenczyk, and Ph.D. students: Marta Bucko, Aleksandra Helwak, Frank King, Grzegorz Kudla, Leszek Lipinski; Grazyna Orleanska, M. Sc. (secretary, appointed in April 15, 2001), technician Wanda Gocal.

Maciej Zylicz, Ph.D.

Education:
1986 Dr. hab., Institute of Biochemistry and Biophysics PAN, Warsaw, Molecular Biology
1979 Ph.D., Medical School, Gdansk, Biochemistry
1977 M.S., University of Gdansk, Physics and Biology

Postdoctoral research training:
1982-1984 Costa Georgopoulos, Ph.D.; University of Utah, Department of Cellular, Viral and Molecular Biology, Salt Lake City, Utah, USA
1979-1981 Karol Taylor, Ph.D.; University of Gdansk, Department of Biochemistry, Gdansk, Poland

Research and academic position:
1999-present Dept. Chairman, Department of Molecular Biology, International Institute of Molecular and Cell Biology in Warsaw, Poland
1994-1999 Dept. Chairman, Department of Molecular and Cellular Biology, Faculty of Biotechnology, University of Gdansk, Poland
1993-1994 Visiting Professor, University of Utah, Medical Center, Oncology, USA
1991 Professor
1991-1994 Dept. Chairman, University of Gdansk, Department of Molecular Biology, Gdansk, Poland
1990-1993 Vice President, University of Gdansk, elected for 3 years
1988-1991 Associate Prof., University of Gdansk, Department of Molecular Biology, Gdansk, Poland
1981-1988 Assist. Prof., University of Gdansk, Department of Biochemistry, Gdansk, Poland

Honors:
1. Member of Polish Academy of Science
2. Member of EMBO
3. Member of American Society of Biochemistry and Molecular Biology
4. Member of Academia Europea

Selected publications since 1997:
*Banecki B., Wawrzynow A., Puzewicz J., Georgopoulos C., Zylicz M. (2001) Structure-function analysis of the zinc binding region of the ClpX molecular chaperone. **J. Biol. Chem.** *in press*;
*Genevaux P., Wawrzynow A., Zylicz M., Georgopoulos C., Kelley W.L. (2000) DjlA is a third DnaK co-chaperone of Escherichia coli and DjlA-mediated induction of colanic acid capsule requires DjlA-DnaK interaction. **J. Biol. Chem.** *in press*;

*Papers marked with an asterisk have the IIMCB affiliation of authors

Gonciarz-Swiatek M., Wawrzynow A., Um, S-J., Learn B.A., McMacken R., Kelly W.L., Georgopoulos C., Sliekers O., Zylicz M. (1999) Recognition, targeting and hydrolysis of the lambda O replication protein by the ClpP/ClpX protease. **J. Biol. Chem.** 274: 13999-14005;

Zylicz M., Wawrzynow A., Marszalek J., Liberek K., Banecki B., Konieczny I., Blaszczak A., Barski P., Jakubkiewicz J., Gonciarz-Swiatek M., Duchniewicz M., Puzewicz J., Krzewska J. (1999) Role of chaperones in replication of bacteriophage lambda DNA. In "Molecular chaperones and folding catalysts" (ed. B. Bukau), **Horwood Academic Publishers**: pp. 295-311;

Zylicz M., Liberek K., Wawrzynow A., Georgopoulos C. (1998) Formation of the preprimosome protects λO from RNA transcription-dependent proteolysis by ClpP/ClpX. **Proc. Natl. Acad. Sci. USA** 95, 15259-15263;

Deloche O., Liberek K., Zylicz M., Georgopoulos C. (1997) Purification and biochemical properties of Saccharomyces cerevisiae Mdj1p, the mitochondrial DnaJ homologue. **J. Biol. Chem**. 272: 28539-28544;

Wawrzynow A., Zylicz M. (1997) The role of various *Escherichia coli* chaperones in DNA replication. In "Guidebook to Molecular Chaperones and Protein Folding Factors" (red. M.-J. Gething), **Oxford: Sambrook & Tooze, Oxford University Press**: pp. 481-489.

Submitted:

*King F., Hohfeld J., Zylicz M. (2001) Co-chaperone regulation of Hsc 70 and Hsp 90 interactions with wild type and mutant p53;

*Zylicz M., King F., Wawrzynow A. (2001) The role of Hsp 70 and its co-chaperone in oncogenesis.

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*Papers marked with an asterisk have the IIMCB affiliation of authors

Description of present research:

Analysis of interactions between human p53 and cytosolic molecular chaperones
(F. King, A. Helwak, A. Wawrzenczyk, A. Wawrzynow, group leader M. Zylicz)

Hsc70, a constitutively expressed Hsp70 heat shock protein, has been shown to participate in many cellular pathways. In mammalian cells, Hsc70 chaperone activity can be differentially regulated by chaperones, such as Hsp40, Bag, Hip (Hsc70 interacting protein), or Hop (Hsc70-Hsp90 organizing protein). Several important cell growth and signaling proteins, such as steroid receptors, cell cycle kinases, and the p53 tumor suppressor protein have been identified as substrates for Hsp40, Hsp70, and Hsp90 molecular chaperones. Previous *in vivo* studies have shown that Hsc70, Hsp40, and Hsp90 exclusively coimmunoprecipitate with mutant p53, but not with wild type p53 protein. We have demonstrated that purified recombinant human molecular chaperones, Hsc70 and Hsp40 can interact with both wild type and mutant p53 in an *in vitro* reconstituted system. Hsc70 forms a complex with wild type and mutant p53 only in the presence of ATP and Hsp40, whereas Hsp40 binds to wild type and mutant p53 in the absence of Hsc70 and ATP. Both human recombinant wild type and mutant p53 protein exist in high molecular weight oligomeric states, which can be dissociated into lower oligomeric species by Hsc70 and Hsp40 in an ATP-dependent reaction. Coimmunoprecipitation studies show that Hsp90 and Hop stabilize complex formation between Hsc70 and mutant p53 but not between Hsc70 and wild type p53. The antiapoptotic factor BAG-1 disrupts complexes formed between Hsc70 and p53 protein. BAG-1 may play a role in regulating p53 stability by inhibiting p53-chaperone complexes and increasing accessibility to proteases, such as calpain or the proteasome pathway. We are currently examining the effects of BAG-1 on heterocomplex formation between mutant p53 and chaperones Hsc70, Hop, and Hsp90. In summary, we are attempting to assemble an *in vitro* model that would help to explain antiapoptotic pathways, involving

Hsp70 and BAG-1, and be used as a tool to develop new cancer therapy agents against cancers caused by mutated p53 protein.

Hsp 70 as a vehicle for protein therapy
(G. Kudla, F. King, G. Mosieniak, M. Zylicz, group leader A. Wawrzynow)

It has been shown recently that the p50 subunit of NF-kB fused to Hsp70 was directed to the nuclear compartment in a cell-type specific fashion (Fujihara and Nadler 1999). Fusion of the human p53 gene with the C-terminal of human Hsp 70 was cloned into a pBAD vector and the gene product was purified from bacteria. The p53-Hsp70 hybrid will be added exogenously to several cancer cell lines or thymocytes isolated from p53 knockout mice, and the import of this hybrid into the cytoplasmic and nuclear compartments will be monitored by use of fluorescence-labeled antibodies. The function of imported p53 hybrids will be tested by the induction of p53-dependent apoptosis.

Cloning and characterization of a novel human protein kinase
(M. Bucko, L. Lipinski, group leader L. Trzeciak)

The study originates from the discovery of a cDNA fragment coding for a part of hypothetical serine/threonine protein kinase, hereby termed GLIK. This fragment was found during studies on the expression of protein kinases in human stomach cancer cell line AGS. Based on the sequence of this fragment we set out to clone the cDNA for the whole protein using mRNA isolated from AGS cells and RACE method. Initial attempts were, however, unsuccessful. In the meantime, a group from Switzerland described the kinase TSSK-3, a third member of a small family of serine/threonine kinases expressed exclusively in testis. The TSSK-3 appears to be a mouse orthologue of the hypothetical human kinase GLIK. We performed HUGO database searches combining TSSK-3 and GLIK sequence information and identified the hypothetical GLIK gene located on chromosome 1. Utilizing the knowledge of the human gene sequence and mouse cDNA sequence we designed primers to human and mouse cDNA and amplified the coding sequences from human and mouse testis cDNA. We have verified the coding sequences and are now in the process of subcloning the cDNAs into expression vectors. Recombinant protein will be then used to study biochemical properties of the kinase and to rise antibodies against it, which are necessary for functional studies. We will also continue RACE studies in order to clone and sequence cDNA ends of human GLIK/TSSK-3.

Search for new Hsp40 homologues
(A. Helwak, M. Zylicz, group leader A. Wawrzynow)

We had purified the first Hsp40 protein from *Escherichia coli* bacteria (J. Biol. Chem. 1985). Subsequently, it has been shown by other laboratories that the amino acid sequence of one domain of this protein, so-called J-domain, is efficiently conserved during evolution. It was also shown that several eukaryotic transcription factors (potential protooncogenes) and DNA replication proteins (including T antigen of SV40) possess the J-domain. It has been demonstrated that Hsp40 is served as an antigen in several autoimmunological diseases. We plan to purify new Hsp40 homologues from *E. coli* and compare their chaperone activity with other, previously identified, Hsp's. This project could potentially lead to identification of a new family of eukaryotic genes, whose protein products are regulated by Hsp70 chaperone.
This year we focus our attention on DjlA. The DjlA is a 30 kDa Type III membrane protein of *E. coli* with the majority, including an extreme C-terminal putative J-domain, oriented towards the cytoplasm. No other regions of sequence similarity aside from the J-domain exist between DjlA and the known DnaK

(Hsp70) co-chaperones DnaJ (Hsp40) and CbpA. In this study, we explored whether and to what extent DjlA possesses DnaK co-chaperone activity and under what conditions a DjlA-DnaK interaction could be important to the cell. We found that the DjlA J-domain can fully substitute for the J-domain of DnaJ using various *in vivo* functional complementation assays. In addition, the purified cytoplasmic fragment of DjlA was shown capable of stimulating DnaK's ATPase in a manner indistinguishable from DnaJ, and, furthermore, DjlA could act as a DnaK co-chaperone in the reactivation of chemically denatured luciferase *in vitro*. DjlA expression in the cell is tightly controlled and even its mild overexpression leads to induction of mucoid capsule. Previous analysis showed that DjlA-mediated induction of the wca capsule operon required the RcsC/RcsB two-component signaling system and that wca induction by DjlA was lost when cells contained mutations in either the *dnaK* or *grpE* genes. We now show using allele-specific genetic suppression analysis that DjlA must interact with DnaK for DjlA-mediated stimulation of capsule synthesis. Collectively, these results demonstrate that DjlA is a co-chaperone for DnaK and that this chaperone/co-chaperone pair is implicated directly, or indirectly, in the regulation of colanic acid capsule.

Laboratory of Molecular Immunology

Staff:
Dr. Jaroslaw Dastych (Head), Urszula Bialek-Wyrzykowska (postdoctoral fellow), Ph.D. students: Violetta Adamczewska, Maciej Olszewski, Dominika Trzaska

Jaroslaw Dastych, Ph.D.

Education:

1992-1995	Postdoctoral training, Fogarty International Fellowship at the Allergic Diseases Section LCI/NIAID/ National Institutes of Health, Bethesda, Maryland, USA
1991	Ph.D. (Dr. Med. Sc.) University School of Medicine in Lodz, Poland, specialty-Medical Biology. Thesis: Induction of Histidine Decarboxylase in the Lungs of Rats Infected with *Nipostrongylus brasiliensis*, the Nematode
1983	M. Sc. University of Lodz, Poland, specialty - Molecular Biology

Professional Experience:

1998-1999	Visiting Scientist, Laboratory of Allergic Diseases/NIAID, National Institutes of Health, Bethesda, Maryland, USA
1995-1998	Acting Head, Allergy Research Section, Department of Biogenic Amines, PAN, Lodz, Poland
1992-1995	Senior Researcher, Allergy Research Section
1985-1992	Research Assistant, Allergy Research Section
1983-1985	Technician, Allergy Research Section

Awards and Grants:
1996, Grant 4 P05A 047 10 from the State Committee for Scientific Research, Poland
1989, Fulbright Scholarship

Membership in Scientific Societies
Polish Society for Experimental and Clinical Immunology
American Academy of Asthma Allergy and Clinical Immunology

Selected publications since 1997:

*Taylor M., Dastych J., Sehgal D., Sundstrom M., Nilsson G., Akin C., Mage R.G., Metcalfe D.D. The kit activating mutation D816V enhances stem cell factor-dependent chemotaxis. **Blood** *in press*;

Dastych J., Wyczolkowska J., Metcalfe D.D. Characterization of α5-integrin-dependent mast cell adhesion following FcεRI aggregation. **Int. Arch. Allergy Immunol.** *in press*;

Wyczolkowska J., Weyer A., Dastych J. (2000) Inhibitory effect of wheat germ agglutinin on mouse mast cell adhesion to fibronectin. **Int. Arch. Allergy Immunol.** 122: 216-23;

*Fukui M., Whittlesey K., Metcalfe D.D., Dastych J. (2000) Human mast cells express the hyaluronic-acid-binding isoform of CD44 and adhere to hyaluronic acid. **Clin. Immunol.** 94: 173-8;

Dastych J., Walczak-Drzewiecka A., Wyczolkowska J., Metcalfe D.D. (1999) Murine mast cells exposed to mercuric chloride release granule associated N-acetyl-β-D-hexosaminidase and secrete IL-4 and TNF-α. **J. Allergy Clin. Immunol.** 103: 1108-1114;

*Papers marked with an asterisk have the IIMCB affiliation of authors

Dastych J., Taub D., Hardison M.C., Metcalfe D.D. (1998) Tyrosine kinase deficient Wv c-kit induces mast cell adhesion and chemotaxis. **Am. J. Physiol.** 44: C1291-C1299;

Dastych J., Hardison M.C., Metcalfe D.D. (1997) Aggregation of low affinity IgG receptors induces mast cell adherence to fibronectin: requirement for the common FcR gamma-chain. **J. Immunol.** 158: 1803-1809;

Hershkoviz R., Preciado-Patt L., Lider O., Fridkin M., Dastych J., Metcalfe D.D., Mekori Y.A. (1997) Extracellular matrix-anchored serum amyloid A preferentially induces mast cell adhesion. **Am. J. Physiol**. 273 (1 Pt 1): C179-C187;

Mekori Y.A., Oh C.K., Dastych J., Goff J.P., Adachi S., Bianchine P.J., Worobec A., Semere T., Pierce J.H., Metcalfe D.D. (1997) Characterization of a mast cell line that lacks the extracellular domain of membrane c-kit. **Immunology** 90: 518-525.

Description of present research:

Research of the Laboratory of Molecular Immunology is focused on the molecular mechanisms of immunotoxicity. Immunotoxic compounds affect the immune system leading to its disregulation and to abnormal immune response that might be harmful to the organism either by an inadequate and inefficient defense against parasites or neoplasm or by an autoimmune response to host antigens or allergic response to common antigens present in the host environment. The concept of immune modulation mediated with environmental factors ("environmental adjuvants") deserves further research as there is a legitimate concern that an increase in the prevalence of allergic diseases in the populations of industrialized countries may be in part related to the exposure of these populations to a growing number of immunotoxicants. This is related to experimental observations that some environmentally derived factors may stimulate the development of a particular type of immune response named Th2, which seems to favor allergic (atopic) response to antigens.

Mast cell mediated modulation of immune response by immunotoxic agents

Mast cells are part of the native immunity, and at the same moment are important effectors for acquired immunity. It is now more and more evident that mast cells perform an important regulatory role in the immune system. Mast cells are accumulated in tissues that are exposed to the outside environment and are capable of releasing large amounts of highly active mediators that may affect other immune cells. There are many experimental observations suggesting that mast cell activation strongly favor the development of Th2 response resulting in production of IL-4 and IL-5, accumulation of eosinophiles and production of IgE. Our working hypothesis is that mast cell derived IL-4 is critical for the processes of immune modulation mediated with some environmental factors. We are now defining the promoter sequences mediating the induction of IL-4 expression in mast cells exposed to heavy metal ions. To this end, murine bone marrow derived mast cells are transfected with series of DNA construct containing the selected 5' upstream DNA sequences of IL-4 gene, and the promoter activity is measured following activation with antigen or exposure to heavy metal ions.

The search for molecular targets of immunotoxic compounds

Some of the elements of stress responding cellular machinery are also engaged in signaling cascades regulating the response to antigens. For example, the JNK/c-Jun pathway is activated with cellular stress and at the same moment this pathway is engaged in signaling through FcεRI, the central mast cell receptor responsible for antigen mediated mast cell activation. This makes the components of the JNK/cJun pathway expressed in mast cells and lymphocytes interesting candidates for the role of molecular targets of action of immunotoxicants. We observed that both antigen and insult with toxic metal ions induces JNK activation and c-Jun phosphorylation in mast cells but these processes have clearly different kinetics.

While antigen-induced JNK phosphorylation quickly returned to baseline, $HgCl_2$-induced JNK phosphorylation remained elevated for several hours. The concomitant activation of mast cells with antigen and heavy metal ions resulted in at least additive effect on the c-Jun phosphorylation. Our attention now is focused on two topics. We want to understand the role of threonine-serine specific phosphatases for heavy metal-induced JNK activation. We would also like to define the effect of $HgCl_2$-mediated accumulation of phosphorylated c-Jun on formation of AP-1 complexes and their consequent interaction with non-consensus AP-1 sites present in IL-4 promoter.

Development of a new technology for immunotoxicity testing

Cytokine expression is a feasible endpoint for immunotoxicity assays. We are developing a new system for testing immunotoxicity *in vitro*, by performing genetic modification of selected cell lines. Two experimental approaches namely, stable transfection and gene targeting are currently used to generate reporter cell lines. The set of selected cell lines - the fluorescent "cell chip" will be assembled and will undergo the process of testing and pre-validation. This includes in particular standardization of the "cell chip" against several "model xenobiotics" (substances already known for their immunotoxic activities observed *in vivo*).

Laboratory of Bioinformatics

Staff:
Dr. med. Leszek Rychlewski (Head), Janusz Bujnicki, M. Sc.

Leszek Rychlewski, M.D., Ph.D.

Scientific Activities:

2000-present Head of the Laboratory of Bioinformatics at the IIMCB

1998-1999 Post Graduate Researcher at the San Diego Supercomputer Center Computational Biology Center. The main project was the development of large databases incorporating complex biological information and a broad set of sequence analysis tools. The goal of the project was the automation of processing of biological information.

1996-1998 Research fellow at the Scripps Research Institute, Department of Molecular Biology. The main project was the development of methods for protein structure and function prediction. The set of methods developed in our group was aimed at identifying potential drug targets from genomic data (or other sequence databases), based on functional inference from their similarity to other known proteins. We developed and utilized secondary structure prediction (NO NAME) and threading methods (GenFold) as well as sophisticated sequence analysis tools (BASIC) to produce and validate function assignments. The procedures were developed for high throughput data analysis and were already used for the structural/functional annotation of several genomes.

1988-1996 Student at the Humboldt - Universität zu Berlin, M.D., 1996, Ph.D. 1998; Two major research projects: experimental (Neurophysiology) and theoretical (Bioinformatics). Although very fundamental in their nature, both activities had important medical implications. The experimental project focused on the influence of drugs (agonists and antagonists of different transmitters or modulators of the signal transduction system of the brain) on central nervous system activity. The theoretical project was aimed to design antibodies with artificially introduced binding sites capable of binding technetium (a metal ion widely used in radiology). The second project was facilitated in collaboration with Schering AG.

Selected publications since 1997:

* Bujnicki J.M., Radlinska M., Rychlewski L. (2001) Polyphyletic evolution of type II restriction enzymes revisited: two independent sources of second-hand folds revealed. **Trends in Biochemical Sciences** *in press*;
* Bujnicki J.M., Rychlewski L. (2001) Identification of a PD - (D/E) XK-like domain with a novel configuration of the endonuclease active site in the methyl-directed restriction enzyme Mrr and its homologs from all three domains of life. **Gene** *in press*;
* Bujnicki J.M., Rychlewski L. (2001) Sequence analysis and structure prediction of aminoglycoside-resistance 16S rRNA:m7G methyltransferases. **Acta Microbiol. Pol.** *in press*;

*Papers marked with an asterisk have the IIMCB affiliation of authors

* Bujnicki J.M., Eloffson A., Fischer D., Rychlewski L. (2001) LiveBench -1: continuous benchmarking of protein structure prediction servers. **Protein Sci.** *in press*;
* Bujnicki J.M., Rychlewski L. (2001) tRNA splicing endonuclease EndA contains two domains similar to the catalytic domains of DNA endonucleases from the unrelated LAGLIDADG and PD-(D/E)XK families, but developed a novel, distinctly localized RNase active site: Implications for the evolution of selfish genetic elements and protein engineering. **Protein Sci.** *in press*;
* Bujnicki J.M., Rychlewski L. (2001) Grouping together highly diverged PD - (D/E) XK nucleases and identification of novel superfamily members using structure-guided alignment of sequence profiles. **J. Mol. Microbiol. Biotechnol.** *in press*;
* Bujnicki J.M., Rychlewski L. (2001) The Herpesvirus alkaline exonuclease belongs to the restriction endonuclease PD-(D/E)XK superfamily: insight from molecular modeling and phylogenetic analysis. **Virus Genes** *in press*;
* Siew N., Elofsson A., Rychlewski L. and Fischer D. (2000) "MaxSub: An automated measure to assess the quality of protein structure predictions". **Bioinformatics** *in press*;
* Bujnicki J.M., Radlinska M., Rychlewski L. (2000) Atomic model of the 5-methylcytosine-specific restriction enzyme McrA reveals an atypical zinc-finger and structural similarity to beta-beta-alpha-Me endonucleases. **Mol. Microbiol.** 37: 1280-1281;
* Bujnicki J.M., Rychlewski L. (2000) Prediction of a common fold for all four subunits of the yeast tRNA splicing endonuclease - implications for the evolution of the EndA/Sen family. **FEBS Letters.** 486: 328-329;
* Bujnicki J.M., Rychlewski L. (2000) Prediction of a novel RNA 2'-O-ribose methyltransferase subfamily encoded by the *Escherichia coli* YgdE open reading frame and its orthologs. **Acta Microbiol. Pol.** 49: 253-261;
* Fischer D., Eloffson A., Rychlewski L. (2000) The 2000 olympic games of protein structure prediction; fully automated programmes are being evaluated vis-a-vis human teams in the protein structure prediction experiment CAFASP2. **Protein Eng.** 13: 667-670;
* Jaroszewski L., Rychlewski L., Godzik A. (2000) Improving the quality of twilight-zone alignments. **Protein Sci.** 9: 1487-1496;
* Pawlowski K., Jaroszewski L., Rychlewski L., Godzik A. (2000) Sensitive sequence comparison as protein function predictor. **Pac. Symp. Biocomput.** 42-53;
* Pawlowski K., Rychlewski L., Reed J.C., Godzik A. (2000) From fold to function predictions: an apoptosis regulator protein BID. **Comput. Chem.** 24: 511-517;
* Jaroszewski L., Rychlewski L., Reed J.C., Godzik A. (2000) ATP-activated oligomerization as a mechanism for apoptosis regulation, Fold and mechanism prediction for CED-4. **Proteins** 39: 197-203;
* Rychlewski L., Jaroszewski L., Li W., Godzik A. (2000) Comparison of sequence profiles. Strategies for structural predictions using sequence information. **Protein Sci.** 9: 232-241;
* Bujnicki J.M. (2000) Phylogenomic analysis of 16S rRNA: (guanine-N2) methyltransferases suggests new family members and reveals highly conserved motifs and domain structure similar to other nucleic acid amino-methyltransferases. **FASEB J.** 14: 2365-2368;
* Bujnicki J.M. (2000) Molecular modeling of M.BssHII methyltransferase. Is circular permutation common to all DNA methyltransferase subfamilies? **Int. J. Biol. Macromol.** 27: 195-204;
* Bujnicki J.M. (2000) Sequence, structural and evolutionary analysis of prokaryotic ribosomal protein L11 methyltransferases. **Acta Microbiol. Pol.** 49: 19-29;
* Bujnicki J.M. (2000) Phylogeny of the restriction endonuclease-like superfamily inferred from comparison of protein structures. **J. Mol. Evol.** 50: 39-44;

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*Papers marked with an asterisk have the IIMCB affiliation of authors

Rychlewski L., Zhang B., Godzik A. (1999) Function insights from structural predictions: Analysis of the *Escherichia coli* genome. **Protein Sci.** 8: 614-24;

Pawlowski K., Zhang B., Rychlewski L., Godzik A. (1999) The Helicobacter pylori genome: from sequence analysis to structural and functional predictions. **Proteins** 36: 20-30;

Zhang B., Rychlewski L., Pawlowski K., Fetrow J.S., Skolnick J., Godzik A. (1999) From fold predictions to function predictions: Automation of functional site conservation analysis for functional genome predictions. **Protein Sci.** 8: 1104-1115;

Bujnicki J.M., Radlinska M. (1999) Molecular phylogenetics of DNA 5mC-methyltransferases. **Microbiol. Pol.** 48: 19-33;

Bujnicki J.M., Radlinska M. (1999) Is the HemK family of putative S-adenosylmethionine dependent methyltransferases a "missing" zeta subfamily of adenine methyltransferases? A hypothesis. **IUBMB Life** 48: 247-250;

Bujnicki J.M. (1999) Comparison of protein structures reveals monophyletic origin of the AdoMet-dependent methyltransferase family and mechanistic convergence rather than recent differentiation of N4-cytosine and N6-adenine DNA methylation. **In Silico Biology** 1: 1-8;

Radlinska M., Bujnicki J.M., Piekarowicz A. (1999) Structural characterization of two tandemly arranged DNA methyltransferases genes from *Neisseria gonorrhoeae* MS11: N-cytosine specific M.NgoMXV and nonfunctional 5-cytosine-type M.NgoMorf2P. **Proteins** 37: 717-728;

Piekarowicz A., Bujnicki J.M. (1999) Cloning of the Dam methyltransferase gene from Haemophilus influenzae bacteriophage HP1. **Microbiol. Pol.** 48: 123-129;

Bujnicki J.M., Radlinska M. (1999) Molecular evolution of DNA-(cytosine-N4) methyltransferases: evidence for their polyphyletic origin. **Nucl. Res.** 27: 4501-4509;

Kelley L.A., MacCallum R.M., Sternberg M., Karplus K., Fischer D., Elofsson A., Godzik A., Rychlewski L., Pawlowski K., Jones D., Bryson K., Rost B. (1999) "CAFASP-1: Critical Assessment of Fully Automated Structure Prediction Methods" **Proteins Suppl.** 3:209-17;

Jaroszewski L., Rychlewski L., Zhang B., Godzik A. (1998) Fold prediction by a hierarchy of sequence and threading methods. **Protein Sci.** 7: 1431-40;

Rychlewski L., Zhang B., Godzik A. (1998) Function and fold predictions for Mycoplasma genitalium proteins. **Fold Des.** 3: 229-238;

Rychlewski L., Godzik A. (1997) Secondary structure prediction using segment similarity. **Protein Eng.** 10: 1143-1153;

Zhang B., Jaroszewski L., Rychlewski L., Godzik A. (1997) Similarities and differences between nonhomologous proteins with similar folds: evaluation of threading strategies. **Fold Des.** 2: 307-317

Current projects involve:

- "Rational Engineering of the metabolism of fatty acids in FLAX (REFLAX) to produce branched chain fatty acids, with potent bio-lubricant properties". This project is financed by the 5th Framework Programme and by the Polish Committee for Scientific Research.
- "Engineering of DNA Methyltransferases" is a project in collaboration with Dr. M. Radlinska (P.I.) and Dr. A. Piekarowicz (co-P.I.) from the Institute of Microbiology, Warsaw University and **L. Rychlewski** (co-P.I.) from IIMCB aimed to investigate and characterize the nature of the specificity of recognition of DNA by proteins form the methyltransferase family.
- "Evolution of RNA Methyltransferases" is a project aimed to investigate the evolution of numerous unrelated RNA methyltransferase families and to predict their structure and function from the amino acid sequence. **Bujnicki J.** (P.I.).

Laboratory of Molecular Neurology

Staff:
Dr. med. Michal Hetman (Head). At this moment his laboratory is at the end of the organization stage. The basic equipment has been purchased, one Ph.D. student has been hired (Agata Gozdz, M.Sc.) and the second one (Agata Kaleta, M.Sc.) will join the lab in July 2001. The third position is vacant and will be filled in the summer 2001.

Michal Hetman, M.D., Ph.D.

Education

1997	Ph.D. in Center of Experimental and Clinical Medicine, Polish Academy of Sciences, Warsaw, Poland with honors
1994	M.D. degree with the second best result
1987-1994	studies on the First Faculty of Medicine, Warsaw Medical School, Warsaw, Poland.

Employment

2000	Head of Laboratory of Molecular Neurology, International Institute of Molecular and Cell Biology in Warsaw, Poland
1997-2000	postdoctoral fellow in Dep. of Pharmacology, University of Washington, Seattle, USA
1995	senior research associate in Tissue Culture Unit, Nencki Institute, Warsaw, Poland
1994 - 1995	intern in CSK WAM University Hospital, Warsaw, Poland

Scientific career

2000	principal investigator in Laboratory of Molecular Neurology, International Institute of Molecular and Cell Biology in Warsaw, Poland
1997-2000	postdoctoral training in Departments of Pharmacology and Environmetal Health, University of Washington, Seattle, USA
1997	Ph.D. in Center of Experimental and Clinical Medicine, Polish Academy of Sciences, Warsaw, Poland (thesis entitled: Cathepsin D gene in mouse and its expression in experimental brain damage)
1996	3 months training in the laboratory of Dr. Henri Rochefort, INSERM Unite 148, Montpellier, France
1996	summer internship in the laboratory of Mariano Barbacid Ph.D., Bristol-Myers-Squibb Pharmaceutical Research Institute, Princeton, NJ, USA
1995-1996	3 months training in Abteilung Biochemie 2, Zentrum Biochemie, Universitaet Goettingen, Goettingen
1993-1997	Ph.D. studies in the laboratory of Leszek Kaczmarek Ph.D., Tissue Culture Unit, Nencki Institute, Warsaw, Poland
1991-1992	Ph.D. studies in the laboratory of Kurt von Figura, Ph.D., Abteilung Biochemie 2., Zentrum Biochemie, Universitaet Goettingen, Goettingen, Germany
1991-1992	training in the laboratory of Jerzy Kawiak Ph.D., Department of Clinical Cytology, Center of Postgraduate Medical Education, Warsaw, Poland
1989-1991	laboratory training in the Department of Histology and Embryology, Warsaw Medical School, Warsaw, Poland

Selected awards and fellowships:

2000 One-year postdoctoral fellowship of American Heart Association
1998 Polish prime-minister prize for distinctive doctoral thesis
1998 Two-years postdoctoral fellowship of American Heart Association
1995 Young Scientist Award of the Foundation for Polish Science
1991 fellowship of the TEMPUS programme of the European Community Commission to support studies at the University of Goettingen

Selected publications since 1997:

Cavanaugh J.E., Ham J., Hetman M., Poser S., Chen Y., Xia Z. (2001) Differential activation of ERK1/2 and ERK5 by neurotrophins, calcium and cAMP. **J. Neurosci.** *in press*;

Hetman M., Zajaczkowski W., Nikolaev E., Quack G., Danysz W., Kaczmarek L. (2000) Behavioural evaluation of long-term neurotoxic effects of NMDA receptor antagonists. **Neurotoxicity Res.** 1: 299-310;

Hetman M., Cavanaugh J.E., Kimelman D., Xia Z. (2000) Role of Glycogen Synthase Kinase 3 beta in neuronal apoptosis induced by trophic withdrawal. **J. Neurosci.** 20: 2567-2574;

Ghatan S., Larner S., Kinoshita Y., Hetman M., Patel L., Xia Z., Youle R.J., Morrison R.S. (2000) p38 MAP kinase mediates bax translocation in nitric oxide-induced apoptosis in neurons. **J. Cell. Biol.**150: 335-347;

Hetman M., Xia Z. (2000) Signaling pathways mediating anti-apoptotic action of neurotrophins. **Acta Neurobiol. Exp.** 60: 531-545;

Hetman M., Kanning K., Cavanaugh J.E., Xia Z. (1999) Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular-signal-regulated kinase and phosphatidylinositol-3 kinase. **J. Biol. Chem.** 274: 22569-22580;

Jaworski, Biedermann I.W., Lapinska J., Szklarczyk A., Figiel I., Konopka D., Nowicka D., Filipkowski R.K., Hetman M., Kowalczyk A., Kaczmarek L. (1999) Neuronal excitation-driven and AP-1-dependent activation of tissue inhibitor of metalloproteinases-1 gene expression in rodent *Hippocampus*. **J. Biol. Chem.** 274: 28106-28112;

Konopka D., Szklarczyk A.W., Filipkowski R.K., Trauzold A., Nowicka D., Hetman M., Kaczmarek L. (1998) Plasticity- and neurodegeneration-linked CREM/ICER mRNA expression in the rat brain. **Neuroscience** 86: 499-510;

Hetman M., Danysz W., Kaczmarek L. (1997) Increased Cthepsin D expression in rat neurons degenerating in response to MK-801. **Exp. Neurol.** 147: 229-237;

Saftig P., Hartmann D., Lullmann-Rauch R., Wolff J., Evers M., Koster A., Hetman M., von Figura K., Peters C. (1997) Mice deficient in lysosomal acid phosphatase develop lysosomal storage in the kidney and central nervous system. **J. Biol. Chem.** 272: 18628-18635.

Research Plans

Death of neurons plays a critical role in development and occurs in many neurological disorders including stroke, trauma, Alzheimer's and Parkinson's diseases (1). In addition, it might also contribute to deleterious effects of many toxins including neurotoxic pesticides, heavy metals and substances of abuse (2-5). A substantial fraction of neuronal death both in development, diseases and neurotoxic conditions seems to occur via an active process of death known as apoptosis (1). Because apoptotic stimuli require intracellular signal transduction to activate the killing machinery of the cell, understanding of mechanisms regulating apoptosis may lead to identification of novel drug targets for neuroprotective therapy. Although there is a large amount of information gathered on apoptosis in non-neuronal cells, apoptosis of neurons is less characterized. Therefore, I decided to focus my research efforts on identification of molecular

mechanisms controlling neuronal apoptosis. My future research would focus on testing three hypotheses resulting from my previous work.

Hypothesis #1: My work showed that activation of GSK3β follows trophic deprivation and that inhibition of GSK3β suppresses trophic-deprivation induced apoptosis (6). I hypothesize that activation of GSK3β mediates neuronal apoptosis not only in trophic deprived neurons but also in response to neurotoxins and disease-related neurological insults. Furthermore, I propose that downstream targets for pro-apoptotic signaling by GSK3β include such proteins as metabolic enzyme pyruvate dehydrogenase, signaling molecule IRS-1 and transcription factors NFAT and CREB. I hypothesize that GSK3β modulating the function of these transcription factors, changes expression of genes involved in apoptosis. Finally, I propose, that GSK3β is a transducer of anti-apoptotic signaling for both PI-3K and ERK1/2 pathways.

Hypothesis #2: I have found that in cortical neurons activation of Erk1/2 can suppress DNA-damage induced apoptosis (7). I propose that ERK1/2 pathway suppresses apoptosis through the interaction with multiple substrates including those, which modulate gene expression. The putative ERK substrates, which can change gene expression affecting neuronal survival, may be CREB, GSK3β, NF-kappaB and p53. I have also observed that DNA-damage can activate ERK1/2 and that this activation is anti-apoptotic (7). Therefore I hypothesize that various forms of stress can activate ERK as a defense mechanism to prevent death of neurons.

Hypothesis #3: I have observed upregulation of Cathepsin D expression in dying neurons (8,9). I hypothesize that increased expression of Cathepsin D is required for neuronal death.

Future research:

There are several important questions I would like to address in the future:

1. What is the role of GSK3β activity in cell death following neurotoxic and disease-related insults in cultured primary cortical neurons and in animal models? Neurotoxins to test would include environmental agents: heavy metals (arsenic, lead, organic mercury compounds) and pesticides (chlorpyrofos) as well as substances of abuse: ethanol, ketamine, PCP and amphetamines. Disease models to test would include β-amyloid exposure (Alzheimer's diseases) and excitotoxicity (stroke, epilepsy).
2. What are the downstream mechanisms of GSK-3β-mediated apoptosis?
3. Are these mechanisms operating *in vivo*?
4. How is GSK-3β activity regulated in neurons?
5. Is Erk activated as defense mechanism in neurotoxic and disease-related insults?
6. What is the mechanism by which ERK suppresses neuronal apoptosis?
7. What is the role of CatD in neuronal apoptosis? How is CatD knock out genotype modifying neuronal apoptosis *in vitro* and *in vivo* ?

Literature:

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Oberto A., Marks N., Evans H. L., Guidotti A. (1996) *J. Pharmacol. Exp. Ther.* 279: 435-442.

Chan G. C., Hinds T. R., Impey S., Storm D. (1998) *J. Neurosci.* 18: 5322-5332.

Ikonomidou C., Bittigau P., Ishimaru M. J., Wozniak D. F., Koch C., Genz K., Price M. T., Stefovska V., Horster F., Tenkova T., Dikranian K., Olney J. W. (2000) *Science* 287: 1056-60.

Hetman M., Cavanaugh J. E., Kimelman D., Xia Z. (2000) *J. Neurosci.* 20: 2567-2574.

Hetman M., Kanning K., Cavanaugh J. E., Xia Z. (1999) *J. Biol. Chem.* 274: 22569-22580.

Hetman M., Filipkowski R. K., Domagala W., Kaczmarek L. (1995) *Exp. Neurol.* 136: 53-63.

Hetman M., Danysz W., Kaczmarek L. (1997) *Exp. Neurol.* 147: 229-237.

Laboratory of Structural Biology
(Joint MPG-PAN Junior Research Group)

Staff:
Dr. Matthias Bochtler (Head), Roman Szczepanowski, M.Sc. (research associate), Renata Filipek, M.Sc. (Ph.D. student) - to be employed in June 2001,

Matthias Bochtler Ph.D.

Education:
1999 Ph.D., Technische Universität München
1995 Diploma, Ludwig Maximilians-Universität München

Postdoctoral research training:
2000 Biotech patenting for Weickmann & Weickmann
1999-2000 Max-Planck-Institut für Biochemie, Martinsried

Selected publications since 1997:

Bochtler M., Hartmann C., Bourenkov G.P., Bartunik H.D., Huber R. (2000) The structure of HslVU and the mechanism of ATP-dependent proteolysis. **Nature** 403: 800-805;

Song H.K., Hartmann C., Ramachandran R., Bochtler M., Behrendt R., Moroder L., Huber R. (2000) Mutational studies on HslU and its docking mode with HslV. **Proc. Natl. Acad. Sci. USA** 97: 14103-14108;

Bochtler M., Ditzel L., Groll M., Hartmann C., Huber R. (1999) The proteasome. **Ann. Rev. Biophys. Biomol. Struct.** 28: 295-317;

Groll M., Heinemeyer W., Jäger S., Ullrich T., Bochtler M., Wolf D.H., Huber R. (1999) The catalytic sites of 20S proteasomes and their role in subunit maturation – A mutational and crystallographic study. **Proc. Nat. Acad. Sci. USA** 96: 10976-10983;

Groll M., Ditzel L., Löwe J., Stock D., Bochtler M., Bartunik H.D., Huber R. (1997) Structure of the 20S proteasome from yeast at 2.4Å resolution. **Nature** 386: 463-471;

Bochtler M., Ditzel L., Groll M., Huber R. (1997) Crystal structure of heat shock locus V (HslV) from *Escherichia coli*. **Proc. Nat. Acad. Sci. USA** 94: 6070-6074;

Current status:
In addition to a generously equipped wet-lab, the structural biology unit will have state of the art equipment for protein crystallography. This includes a high brilliance rotating anode generator (RU-H3RHB from MSC), Max-Flux confocal optical mirrors, a MAR345 low noise X-ray detector and a cryosystem. The unit will be located in the basement of the IIMCB building. Reconstruction work is currently getting underway (April 2001) to deal with the excess heat that is generated as a byproduct by the X-ray generator. Delivery of the generator is expected for June 2001. This date will be the official starting date for the group. Two extra positions in the group for Ph.D. students will be filled within two months. The wet lab work has started already.

Description of present and planned research:
In eukaryotic cells, most intracellular proteins (Hershko, 1992) and at least some proteins from the endoplasmic reticulum (Hiller, 1996) and the plasma membrane (Hicke, 1997) are degraded by the ubiquitin proteasome system. The large diversity of substrates makes the proteasome-ubiquitin system a player in many cellular processes, including stress response (Finley, 1987), cell cycle control (King, 1996;

Pagano, 1997) and metabolic adaptation (Mitsch, 1996). In mammals, the peptides that result from proteasome-mediated degradation of cytosolic proteins are translocated through the ER-membrane and exported on major histocompatibility complex (MHC) class I molecules to the cell surface, where they are displayed to cytotoxic T lymphocytes, explaining the importance of the proteasome ubiquitin system in the specific immune response (York, 1996, Koopmann, 1997). Not surprisingly, defects in the ubiquitin-proteasome pathway have been implicated in the pathogenesis of a broad range of human diseases. A 1999 review lists several forms of malignancies, a number of genetic diseases (cystic fibrosis, Angelman's syndrome, Liddle syndrome), defects in immune surveillance and muscle wasting (Schwarz, 1999). The list has grown since, with the important addition of Parkinson's disease (Leroy, 1998).

My interest in the system has so far been focused on the structural biology of proteasomes (Bochtler, 1999), and more particularly on HslVU, a prokaryotic model system for the 26S proteasome. In Martinsried, we have recently solved the structure of a complex of HslV and HslU (Bochtler, 2000). This structure provided the first experimental demonstration that Clp/Hsp100 proteins are members of the AAA-superfamily of ATPases. In addition, the structure clearly demonstrated how ATP-binding and release drive conformational motion in AAA-proteins, thus explaining at least in part how the chaperone activity of these proteins depends on ATP-hydrolysis. Efforts to block the ubiquitin-proteasome pathway have so far focused on the inhibition of 20S proteasomes. Many groups have generated proteasome inhibitors, including peptide aldehydes, α-keto-aldehydes, α-keto-acids, boronic esters, boronic acids, vinyl sulfones and α'β'-epoxyketones. A monofunctional peptidyl boronic acid proteasome inhibitor, PS 341, is currently entering phase II clinical trials as an anticancer agent. This is strong support for the concept that inhibitors of the ubiquitin proteasome system have potential in the clinic.

However, because of the broad role of proteasomes in many cellular processes, inhibitors of the proteasome itself are expected to have many side effects. The unifying concept for the research group at the IIMCB is the assumption that compounds with fewer side effects should be targeted not to the executioner in the pathway, i.e. the proteasome, but to the specificity component of the system, i.e. the ubiquitin system. Plans include work on ubiquitin activating enzymes, that ligate ubiquitin, a marker for protein degradation, to the activating enzyme as a thioester (Ciechanover, 1981). The work will touch on ubiquitin conjugating enzymes that act as transient storage units for activated ubiquitin. The major focus of the work will be ubiquitin ligases that impart specificity to protein degradation and catalyse the conversion of a ubiquitin thioester to a ubiquitin isopeptide bond (Scheffner, 1995; Haas, 1997). An additional interest of the group is in ubiquitin hydrolases and ubiquitin specific proteases, that counteract the action of these ubiquitinating enzymes (Wilkinson, 1997). Mechanistic aspects of thioester formation and isopeptide bond hydrolysis will also be studied in related protein conjugation systems such as the Apg12 conjugation system, the Rub conjugation system and the Smt3/SUMO conjugation system (Jentsch, 1998). It is hoped that the x-ray structures of enzymes in these pathways stimulate the design of small molecule ligands for these enzymes.

Hershko A., Ciechanover A. 1992. *Annu. Rev. Biochem.* 61:761–807
Hille, M. M., Finger A., Schweiger M., Wolf D. H. 1996. *Science* 273:1725–28
Hicke L. 1997. *FASEB J.* 11:1215–26
Finley D., Özkaynak E., Varshavski A. 1987. *Cell* 48:1035–46
King R.W., Deshaies R.J., Peters J.-M., Kirschner M. W. 1996. *Science* 274:1652–58
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Koopmann J.-O., Hämerling G.J., Momburg F. 1997. *Curr. Opin. Immunol.* 9: 80–88
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Bochtler M., Ditzel L., Groll M., Hartmann C., Huber R. (1999). *Ann. Rev. Biophys. Biomol. Struct.* 28: 295-317

Bochtler M., Hartmann C., Bourenkov G.P., Bartunik H.D., Huber R. (2000). *Nature* 403: 800-805

Ciechanover A., Heller H., Katz-Etzion R., Hershko A. 1981. *Proc. Nat. Acad. Sci. USA* 78: 761-765

Scheffner M., Nuber U., Huibregtse J.M. 1995. *Nature* 373: 81–83

Haas A.L., Siepmann T.J. 1997. *FASEB J.* 11: 1257–68

Wilkinson K.D. 1997. *FASEB J.* 11: 1245–56

Jentsch S., Ulrich H.D. 1998. *Nature* 395: 321-323

Laboratory of Neurodegeneration

Staff:
Jacek Kuznicki (Head), Cezary Zekanowski, Ph.D., Sanne Mikkelsen, M.Sc., Tomek Rudka (student), Malgorzata Mossakowska, Ph.D. (Centenarian Programme), Aleksandra Szybinska, M.Sc. (Cell and DNA bank in Centenarian Programme)

Jacek Kuznicki Ph.D.

Education:

1993	Professorship, nominated by the President of RP
1987	Habilitation and Docent degree, Nencki Institute
1980	Ph.D., Nencki Institute of Experimental Biology, Warsaw, Poland
1976	Master of Science, University of Warsaw, Poland

Employment:

1998 - present	Deputy Director at the International Institute of Molecular and Cell Biology in Warsaw, Acting Director since January, 1999
1996 - present	Laboratory Head at the Nencki Institute
1992 - 1995	Visiting Professor at the NIMH, Lab of Clinical Science, Bethesda, U.S.A.
1991 - 1992	Deputy Director (Scientific Director), professor at the Nencki Institute
1986 - 1992	Laboratory Head, Associate Professor at the Nencki Institute
1984 - 1985	Research Associate at the Nencki Institute
1981 - 1984	Visiting Fellow, NHLBI, Lab of Cell Biol. (Dr. E.D. Korn), Bethesda, U.S.A
1980 - 1981	Postdoctoral Fellow at the Nencki Institute
1976 - 1980	Ph.D. student at the Nencki Institute

Recent grants

2001	research grant from State Committee for Scientific Research (KBN) on "The search for a functional bio-marker of familial Alzheimer disease (FAD) - identification of the protein that changes affinity to presenilin 1 as a result of its mutation" (3 years)
2001	KBN "ordered grant" on "Genetic and environmental mechanisms of healthy longevity in Polish Centenarians" (3 years)
2001	UNESCO grant to support studies on Polish Centenarians (1 year)
1999	Grant from PHARE Sci-Tech, Centre of Excellence for Studies on Mechanisms of Neurodegeneration, its 2 year continuation will be provided by a KBN as an "ordered grant"
1998	Grant from Maria Sklodowska-Curie FUND II on "Mechanisms of calretinin gene expression in neurons and cancer cells" (4 years)
1998	Grant from the International Center for Genetic Engineering and Biotechnology on "Structure – function analysis of calretinin - a neuronal calcium binding protein" (3 years)

Present research interests in the form of key words:
Alzheimer disease, neurodegeneration, aging and longevity, protein structure-function analysis, calcium regulation and homeostasis, calcium-binding proteins, cell specific protein expression, transcription regulation and gene promoters.

Present projects:
The search for a functional bio-marker of familial Alzheimer disease (FAD) - identification of the protein that changes affinity as a result of presenilin mutations.

Mutations in presenilin genes are casually linked to familial Alzheimer's disease (FAD). These mechanisms appear to involve perturbed Ca^{2+}-signaling that may be the pre-symptomatic stage of FAD. The hypothesis is proposed that mutated presenilins bind to a different protein(s) than wild type ones, and such a protein is either a Ca^{2+}-binding protein, or its target. If so, this change perturbs Ca^{2+}-signaling and leads to secondary symptoms of FAD. We will compare the pattern of proteins bound to mutated presenilins in the lymphocytes of FAD patients with proteins bound to presenilins in lymphocytes of age-matching patients and non-dementia Centenarians.

Polish Centenarians Programme "Studies on environmental and genetic aspects of longevity" (http://www.iimcb.gov.pl/centenarians/centenarians.htm). This Programme started at IIMCB in autumn 1998 thanks to the initiative and effort of Dr. Jacek Kuznicki and considerable support from doc. dr. hab. Ewa Sikora (Nencki Institute), Doc. dr. hab. M.D. Maria Barcikowska (CMDiK) and Dr. hab., M.D. Krzysztof Galus (Gerontology Clinic, CMKP, Warsaw). Its aims, and preliminary data were described: Mossakowska M., Puzianowska-Kuznicka M., Barcikowska M., Chiron-Jouan S., Czyzewski K., Derejczyk J., Franceschi C., Gabryelewicz T., Galus K., Grodzicki T., Gross R., Klich-Raczka A., Luczywek E., Passeri G., Pfeffer A., Pruszynski J., Radziszewska E., Sikora E., Sosnowski M., Styczynska M., Wasiak B., Wieczorowska-Tobis K., Zyczkowska J., Kuznicki J. "Program i badania polskich stulatkow "PolStu99" - poszukiwanie czynnikow sprzyjajacych dlugowiecznosci" 2000, Gerontologia 8 (4) 35-39.

The scientific aim of the Programme is to collect information on the environmental determinants of healthy aging in Polish centenarians and to provide material to study several aspects of longevity including the search for genetic determinants. The bank of DNA, RNA, and immortalized lymphocytes consists of samples from Centenarians, healthy subjects, and Alzheimer disease patients. The social aim of the project is to get the public attention to the aging population, living conditions of old people and to attract young Polish medical doctors into gerontology.

The multidisciplinary Pogramme "Environmental and genetic factors of Polish centenarians' longevity", in which 22 research groups from different labs in Poland will take part, has been accepted for funding in 2001- 2004 by KBN. The package is coordinated by Dr. J. Kuznicki, the organization of medical examination, blood analysis and database by Dr. M. Mossakowska, and it consists of 7 original projects:

- Health status evaluation of Polish centenarians, including cardiac system, Dr. Tomasz Grodzicki
- The neurological and neuropsychological status of Polish centenarians with particularly estimation of dementia risk factors, extrapyramidal function and postural stability, Dr. Anna Pfeffer
- Psychological aspects of functioning in Polish centenarians, Dr. Elzbieta Szelag
- Evaluation of neuroendocrine system, mineral balance and osseous system, Dr. Boguslawa Baranowska
- Immune system of Polish centenarians including the function of CD8+CD28 - subpopulation of T lymphocytes, Dr. Ewa Sikora
- Evaluation of antioxidant status in Polish centenarians, Dr. Barbara Klapcinska
- Establishment of DNA, RNA and immortalized lymphocytes bank. Study on chromosomal aberrations and polymorphism of genes connected with ageing, Dr. Michal Witt.

Selected publications since 1997:

Nowotny M., Bhattacharya S., Filipek A., Krezel A.M., Chazin W., Kuznicki J. (2000) Characterization of the interaction of calcyclin (S100A6) and calcyclin-binding protein. **J. Biol. Chem.** 275: 31178-31182;

Jastrzebska B., Filipek A., Nowicka D., Kaczmarek L., Kuznicki J. (2000) Calcyclin (S100A6) binding protein (CacyBP) is highly expressed in brain neurons. **J. Histochem. Cytochem.** 48: 1195-1202;

Lesniak W., Jezierska A., Kuznicki J. (2000) Upstream stimulatory factor is involved in the regulation of the human calcyclin (S100A6) gene. **Biochim. Biophys. Acta** 1517: 73-81;

Lesniak W., Swart G.W.M., Bloemers H.P.J., Kuznicki J. (2000) Regulation of cell specific expression of calcyclin (S100A6) in nerve cells and other tissues. **Acta Neurobiol. Exp.** 60: 569-575;

Palczewska M., Groves P., Kuznicki J. (1999) Use of Pichia pastoris for expression, purification and characterization of rat calretinin "EF-hand" domains. **Protein Expres. Purif**. 17: 464-465;

Billing-Marczak K., Przybyszewska M., Kuznicki J. (1999) Measurements of [Ca^{2+}] using fura-2 in glioma C6 cells expressing calretinin with GFP as a marker of transfection: no Ca^{2+}-buffering provided by calretinin. **Biochem. Biophys. Acta** 1449: 169-177;

Filipek A., Kuznicki J. (1998) Molecular cloning and expression of a mouse brain cDNA encoding a novel protein target of calcyclin. **J. Neurochem.** 70: 1793-1798;

Groves P., Finn B.E., Kuznicki J., Forsen S. (1998) A model for target protein binding to calcium-activated S100 dimers. **FEBS Lett.** 421: 175-179;

Strauss K.I., Kuznicki J., Winsky L., Hammer M., Jacobowitz D.M. (1997) The mouse calretinin gene promoter region: structural and functional components. **Mol. Brain. Res.** 49: 175-187.

Laboratory of Developmental Neurobiology
(to be organized)

Staff:
Jean-Marc Matter Ph.D. (Head)

This laboratory will be established upon arrival of Dr. Matter to Poland in the fall 2001. Dr. Matter was selected together with Dr. Hetman as the leader of a separate laboratory. His research interest focuses on transcriptional codes specifying neuronal identity during neurogenesis in vertebrates. His laboratory space has been furnished. The organization of the laboratory will start in September.

Jean - Marc Matter Ph.D.

Education and professional experience:

1993-present	"Charge d' enseignement et de Recherche", Department of Biochemistry University of Geneva
1988-1993	"Maitre assistant", Dr. Marc Ballivet's laboratory, Department of Biochemistry, University of Geneva
1988 (IX-XI)	Invited fellow, Dr. Mark Fishman's laboratory, Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
1987	Research Associate, W.M. Cowan's laboratory, Center for studies of Higher Brain Function, Washington University, School of Medicine, St. Louis, MO, USA
1984-1986	Post-doctoral fellow with Dr. W. Maxwell Cowan, Developmental Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, USA
1980-1983	Thesis work with Dr. Roger Weil, Department of Molecular Biology, University of Geneva. Ph.D. degree awarded in November 1983
1979	Postgraduate training programme in Molecular Biology, University of Geneva
1974-1978	Biology Major at University of Geneva

Fellowships and grants

1997-2001	Swiss NSF grant 3100-046866.96: "The neuronal nicotinic acetylcholine receptors: structure and transcriptional regulation".
1992-1996	Swiss NSF grant 31-30861.91: "The neuronal nAChR genes"
1988	Grant from the "Societe Academique", Geneva
1988-1991	Swiss NSF grant 3. 169.0.88: "The neuronal nAChR genes"
1984-1986	Post-doctoral fellow of the Swiss National Science Foundation
1972	Winner of the contest "la Science appelle les jeunes"
1973	Toppalli's Prize, College of Geneva

Selected publications since 1997:

Erkman L., Matter J.-M., Matter-Sadzinski, L., Ballivet M. (2000) Nicotinic acetylcholine receptor gene expression in developing chick autonomic ganglia. **Eur. J. Pharm.** *in press*;

Matter J.-M., Ballivet M. (2000) Gene structure and transcriptional regulation of the neuronal nicotinic acetylcholine receptors. **Handbook of Pharmacology** *in press*;

Matter J.-M., Matter-Sadzinski L., Roztocil T., Hernandez M.-C., Couturier S., Ong M.-T. and Ballivet M. (1998) On the transcriptional regulation of neuronal nAChR genes. **J. Physiol. (Paris)** 92: 245-248;

Roztocil T., Matter-Sadzinski L., Gomez M., Ballivet M., and Matter J.-M. (1998) Functional properties of the neuronal nicotinic acetylcholine receptor β3 promoter in the developing central nervous system. **J. Biol. Chem.** 273: 15131-15137;

Fucile S., Matter J.-M., Erkman L., Ragozzino D., Barabino B., Grassi F., Alema S., Ballivet M., and Eusepi F. (1998) The neuronal α6 subunit forms functional heteromeric acetylcholine receptors in human transfected cells. **Eur. J. Neurosci.** 10: 172-178;

Roztocil T., Matter- Sadzinski L., Alliod C., Ballivet M. and Matter J.-M. (1997) NeuroM, a neural helix-loop-helix transcription factor, defines a new transition stage in neurogenesis. **Development** 124: 3263-3272.

Submitted:

Matter-Sadzinski L., Ong M.T., Hernandez M.-C., Alliod C., Ballivet M., and Matter J.-M. (2001) Interactions of different bHLH proteins specify neurotransmitter receptor identity of ganglion cells in the retina.

Research interests

Transcriptional codes specifying neuronal identity during neurogenesis in vertebrates

The assembly neural circuits in the vertebrate nervous system is anticipated by the orderly differentiation of a vast array of diverse neurons whose phenotypes include such essential traits as membrane excitability and neurotransmitter specificity. Several transcription factor families play crucial parts in neurogenesis and among them the basic helix-loop-helix (bHLH) factors are important regulators of neuronal birthdate and identity. In Drosophila, bHLH factors encoded by the achaete-scute and atonal genes are the main intrinsic determinants of neural fate and numerous achaete-scute (ASH) and atonal (ATH) homologues have been identified in vertebrates. They are sequentially expressed and there is evidence that the products of the early or upstream genes may be required for the expression of the late or downstream genes (1).
The coordinated expression of multiple bHLH factors have a central role in specifying particular neuronal phenotypes but little is known about the interactions taking place between various members of the neuronal bHLH family, as well as those between bHLH factors and terminal differentiation genes (2). By using the retina as model system to study molecular basis of neurogenesis, we have clarified several aspects of the genetic circuitry underwriting the ganglion cell phenotype in the retina (3,4,5). We have shown that expression of the β3 subunit of a neuronal nicotinic acetylcholine receptor subtype is confined to the ganglion cells, which it may impart specific neurotransmitter receptivity and membrane excitability. The promoter conferring stringent neuronal specificity upon the β3 gene has been characterized in detail and shown to be under the control of bHLH proteins (6,7). The promoter, whose activation is an early marker of ganglion cell differentiation (5), is therefore a useful tool identify the transcription factors specifying ganglion cell identity. We have shown that the β3 promoter is under the direct and specific control of the *atonal* homologue cATH5 (8). To characterize the cascade of gene regulations leading to β3 expression, we have isolated the cis-regulatory domain of the cATH5 gene. We have determined that it is activated by Ngn2, NeuroM, NeuroD and cATH5 itself, and strongly inhibited in a dominant-negative mode by cASH1. These results were fully corroborated by the expression patterns of the corresponding genes in the course of retina development. They suggest that the cATH5 promoter integrate the effects of several sequentially expressed bHLH factors to coordinate the specification of ganglion cell identity within the overall programme of retinogenesis. They provide the first demonstration that terminal differentiation genes can be regulated directly by a transcription factor, which may act as a coupling device between transcriptional pathways controlling generic aspects of neurogenesis and those that regulate specific features of central neurons (8).

CATH5 and β3 nAChR are among the few *neuronal transcription factor* and *neuron-specific terminal differentiation genes* for which the natural transcription factors controlling expression in central neurons have been identified (8). This knowledge should enable us to address several important questions about mechanisms of gene regulation in retinal neurons:

(I) The cATH5 transcription factor is specifically expressed during early retinogenesis and we will use the inducible activity of the aATH5 promoter in retinal cells as a sensitive and specific functional assay to identify extrinsic and intrinsic factors that initiate retina development in vertebrates.

(II) Activity of the cATH5 promoter in retinal cells can be detected in vivo by using the GFP as a reporter gene. We are developing techniques based on the use of GFP imaging and single-cell RT-PCR to identify regulatory genes expressed by specific subset of retinal progenitors.

(III) The cATH5 and β3 promoters contain different functional E-box elements and they are regulated by different neuronal bHLH transcription factors. Combining promoter mutagenesis and functional analysis of chimerical bHLH transcription factors, we have started an extensive research programme to define the molecular basis of transcription factor selection in central neurons. Such works are essential to learn how to design specific and neuronal promoters and transcription factors to be used in the future for gene therapy purposes.

Educational activities

Utrecht University Ph.D. Programme

The Utrecht University Ph.D. programme is based on an agreement between the Polish Network for Cell and Molecular Biology UNESCO/PAN and the Utrecht University (The Netherlands). This is a part of the research collaboration programme initiated by Prof. Wilem Gispen to facilitate the exchange of scientific information and ideas among Polish and Dutch scientists and graduate students and allow for short-time research visits of the staff members and their students from Poland to Utrecht and vice versa. The Ph.D. programme itself offers three 4-year doctoral positions (based on financial support of 55.000 Dutch guilders a year). The Ph.D. thesis will be defended in front of the dissertation committee of the Medical Faculty of Utrecht, Utrecht University. As a result of publicly advertised competition three students were accepted: Marta Bucko (M. Zylicz's lab), M. Olszewski (J. Dastych's lab) and K. Starowicz (R. Przewlocki's lab, Institute of Pharmacology, PAN, Cracow).

Postgraduate School of Molecular Medicine

Postgraduate School of Molecular Medicine: Medical Universities in Warsaw, Poznan, Szczecin, Gdansk as well as the International Institute of Molecular and Cell Biology, the Nencki Institute and the Foundation for Experimental and Clinical Oncology have founded the Postgraduate School of Molecular Medicine (SMM). The main goal of the School is to offer a new postgraduate Ph.D. programme in the field of molecular medicine, which is addressed to medical, biology and pharmacy students in Poland.
SMM is affiliated with the Medical University of Warsaw, which is responsible for administration of the school. According to its by-laws, the School is managed by the Director and the Scientific Council elected by the founding institutions. SMM admits students (up to 10 persons per year) for the 4-year Ph.D. programme. The candidates are requested to present a scientific programme of their Ph.D. study, scientific merit of which is carefully evaluated by the Recruitment Committee of SMM as well as independent referees in Poland and abroad. Three groups of students were accepted so far: one in 1998, 1999 and 2000. Successful candidates accomplish their scientific programmes, under supervision of their tutors, in different laboratories throughout Poland. The members of SMM Scientific Council annually evaluate students' progress. The tutorial programme offered to the students include theoretical (lectures, seminars) and practical courses (laboratory sessions) in selected topics of modern molecular biology and medicine. Each SMM student is awarded a stipend (full or supplemental). Furthermore, SMM helps students to participate in short-term scientific training in leading Polish and foreign laboratories. SMM activities are supported by subsidies from the Polish Ministry of Health and founding institutions. Additional generous help also came from the French government that offered financial support for covering 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.

Computer Network

The IIMCB computer network is implemented over a structured network of copper 5th category cable with 250 entry active points (the max. number of IP devices on the net). Active elements of the network are: two optic fiber transceivers and two 3Com 24-ports switches Ethernet 10/100 Mb/s. There are about 30 workstations, notebooks and pads in the network protected by the local firewall (the second is planed in the future) operating under Windows NT/2000, Linux, Solaris and Mac Os. We have 6 Institute servers (ordinary PC workstations) used for mail, intranet, www access, dns, dhcp, applications/files, remote access, proxy and firewall and they operate under Win NT 4.0/ W2K and Linux. 5 printers work as network devices and 2 modems connect computers with the telephone system allowing users remote access from home. This year we have implemented a new optic fiber connection with Nencki Institute in order of integration of services and facilities between two Institutes. The next year we plan to implement a connection to the rest of the Ochota campus through ATM 155/625 Mb/s and to upgrade a local network to the Gigabit Ethernet. A plan for future purchases contains: a server cluster with a common mass storage for applications/files server for biomedical databases and centenarians project database under SQL.

Financial Statements

INCOME AND EXPENSES OF THE INTERNATIONAL INSTITUTE OF MOLECULAR AND CELL BIOLOGY I – XII 2000

I.	**BUILDING EXPLOITATION**	**266 322,09**
1.	Electricity, hot water and other utilities	132 879,56
2.	Conservation, renovation	70 663,77
3.	Cleaning, security	25 716,20
4.	Mail, telephone bills	37 062,56
II.	**RESEARCH and OPERATION COSTS**	**1 145 350,43**
1.	Equipment, furniture, computers	63 375,65
2.	Laboratory research equipment	163 140,59
3.	Materials	282 448,64
4.	Depreciation	153 881,99
5.	Taxes	95 575,03
6.	Other	386 928,53
III.	**SALARIES**	**954 189,17**
1.	Salaries	793 109,73
2.	Contribution for National Insurance Scheme	110 086,97
3.	Year reward	50 992,47
	TOTAL COSTS	**2 365 861,69**
	BUDGETARY DONATION	1 292 000,00
	OTHER DONATIONS	919 117,70
	INTEREST	33 736,79
	TOTAL INCOME	**2 244 854,49**
	LABORATORY RESEARCH EQUIPMENT – INVESTMENT COSTS	**301 210,86**

Staff at IIMCB

(as of May 10th, 2001)

Name	Function	Employer
1. Angelo Azzi	Director	(IIMCB)
2. Jacek Kuznicki	Acting Director	(IIMCB, 1/2)
3. Michal Witt	Deputy Director	(IIMCB, 1/2)
4. Zbigniew Przygoda	Administration Manager	(IIMCB, 1/2)
5. Andrzej Sliwowski	Network Manager	(IIMCB, full)
6. Hanna Michalska	Chief of Accounting	(IIMCB, 1/2)
7. Iwona Marchewka	Accounting	(IIMCB, 1/2)
8. Agnieszka Ziemka	Secretary	(IIMCB, full)
9. Dorota Lupa	Secretary	(IIMCB, full)
10. Ewa Blazewicz	Secretarial assistant	(IIMCB, ¾)
Department of Molecular Biology:		
11. Maciej Zylicz	Head	(IIMCB+UNESCO fellowship)
12. Alicja Wawrzynow	Vice Head	(IIMCB+UNESCO fellowship)
13. Grazyna Mosieniak	Postdoctoral Fellow	(Nencki Institute)
14. Agnieszka Wawrzenczyk	Postdoctoral Fellow	(Nencki Institute)
15. Leszek Trzeciak	Postdoctoral Fellow	(Nencki Institute)
16. Frank King	Ph.D. Student	(IBB + Kosciuszko Foundation)
17. Marta Bucko	Research Assistant	(Utrecht fellowship)
18. Grzegorz Kudla	Research Assistant	(SMM)
19. Aleksandra Helwak	Research Assistant	(IBB)
20. Leszek Lipinski	Research Assistant	(IBB)
21. Wanda Gocal	Technician	(IIMCB, full)
22. Grazyna Orleanska	Secretary	(IIMCB, full)
Laboratory of Molecular Immunology:		
23. Jaroslaw Dastych	Head	(IIMCB, full)
24. Urszula Bialek-Wyrzykowska	Postdoctoral Fellow	(Vth FP EU)
25. Dominika Trzaska	Research Assistant	(IIMCB, full)
26. Violetta Adamczewska	Research Assistant	(IIMCB, full)
27. Maciej Olszewski	Research Assistant	(Utrecht fellowship)
28. Vacant	Research Assistant	(IIMCB)
Laboratory of Bioinformatics:		
29. Leszek Rychlewski	Head	(IIMCB, 1/2)
30. Janusz M. Bujnicki	Research Assistant	(Volunteer)
31. Zuzanna Stasinska	M.Sc. Student	(Volunteer)

32. Marcin Feder	M.Sc. Student	(Volunteer)
33. Jakub Pas	M.Sc. Student	(Volunteer)

Laboratory of Molecular Neurology:

34. Michal Hetman	Head	(IIMCB, full)
35. Agata Gozdz	Research Assistant	(IIMCB, full)
36. Agata Kaleta	Research Assistant	(IIMCB, full, since July 2001)
37. Vacant	Research Assistant	(IIMCB)

Laboratory of Structural Biology (Joint MPG-PAN Junior Research Programme):

38. Matthias Bochtler	Head	(Max-Planck)
39. Roman Szczepanowski	Research Assistant	(Max-Planck)
40. Renata Filipek	Research Assistant	(Max-Planck)
41. Vacant	Research Assistant	(Max-Planck)

Laboratory of Neurodegeneration:

42. Jacek Kuznicki	Head	
43. Cezary Zekanowski	Postdoctoral Fellow	(IIMCB, full)
44. Sanne Mikkelsen	Research Assistant	(PAN-Danish fellowship)
45. Tomasz Rudka	M.Sc. Student	(Volunteer)
46. Malgorzata Mossakowska	Centenarians Project	(IIMCB, full)
47. Aleksandra Szybinska	Cell and DNA Bank	(IIMCB, full)

Laboratory of Developmental Neurobiology:

48. Jean-Marc Matter	Head	(IIMCB/Swiss grant)
49. Monika Puzianowska-Kuznicka	Postdoctoral Fellow	(to be employed in September 2001)
50. Vacant	Research Assistant	(IIMCB)

Utrecht University fellowship (at the Institute of Pharmacology PAN in Cracow):

51. Katarzyna Starowicz	Research Assistant