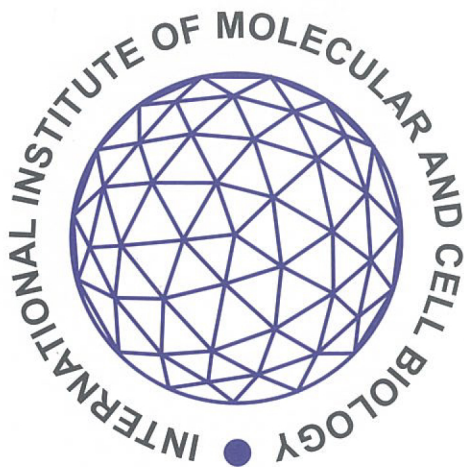




**International Institute
of Molecular and Cell Biology
in Warsaw**

Annual Report 2001

Contents:

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Jacek Kuznicki

Deputy Director

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Map of the Ochota Campus

International Institute of Molecular and Cell Biology in Warsaw is located at the Ochota Campus - the largest biomedical centre in the Central and Eastern Europe

1 International Institute of Molecular and Cell Biology in Warsaw

Polish Academy of Sciences

- 2 Nencki Institute of Experimental Biology
- 3 Medical Research Center
- 4 Institute of Biochemistry and Biophysics
- 5 Institute of Biocybernetics and Biomedical Engineering

6 Medical University of Warsaw

- 7 Faculty of Pharmacy
- 8 Hospital
- 9 President's office

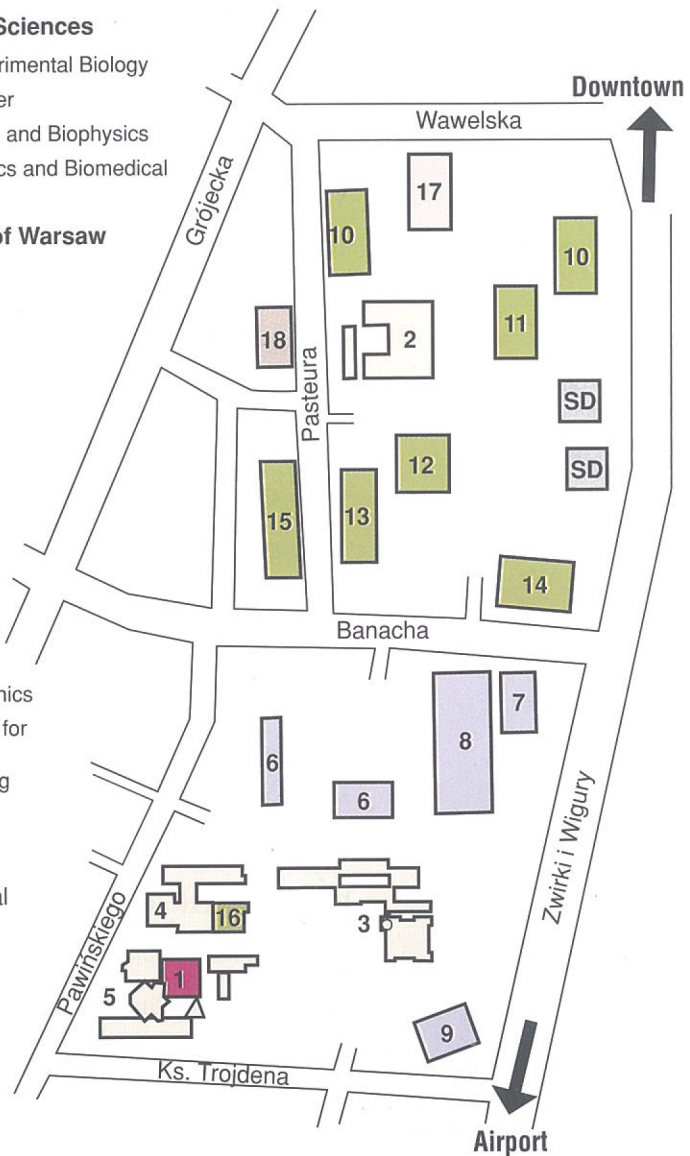
Warsaw University

- 10 Faculty of Chemistry
- 11 Faculty of Biology
- 12 Heavy Ion Laboratory - cyclotron
- 13 Faculty of Geophysics
- 14 Faculty of Geology
- 15 Faculty of Mathematics, Informatics and Mechanics
- 16 Interdisciplinary Centre for Mathematical and Computational Modeling

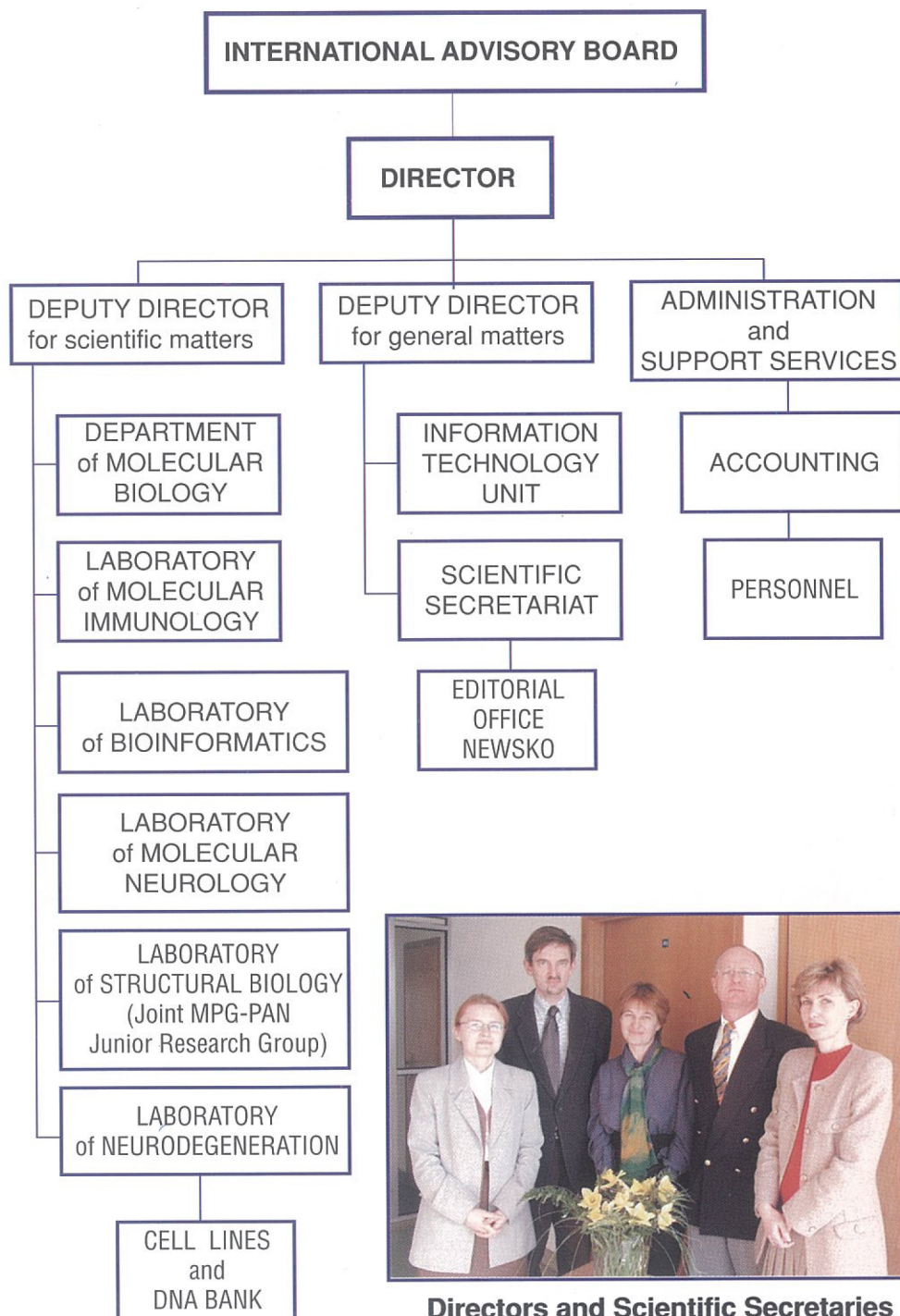
17 Oncology Hospital

18 Pulmonology Hospital

SD Student Dormitories



Structure of the Institute



International Advisory Board

(1999 – 2001 term)

Chairman: Maciej J. Nalecz

Vice Chairmen: Nathan Nelson and Ryszard Przewlocki

Members:

Ken-ichi Arai, Director, Institute of Medical Sciences, University of Tokyo; Chairman, International Molecular Biology Network for Asia and the Pacific Rim (A-IMBN); 4-6-1, Shiroganedai, Minato-ku, Tokyo 108, Japan

Angelo Azzi*, Director, Institute of Biochemistry and Molecular Biology, University of Berne, Switzerland; Director (till April, 2001), International Institute of Molecular and Cell Biology in Warsaw, Poland; Chairman, UNESCO Global Network for Molecular and Cell Biology (MCBN), Buehlstrasse 28, 3012 Berne, Switzerland

Frank Gannon, Executive Director, European Molecular Biology Organization, Postfach 10 2209, D-69012 Heidelberg, Germany

Willem H. Gispen, Director, Rudolf Magnus Institute for Neurosciences; Rector, University of Utrecht, Universiteitsweg 100, 3584 CG Utrecht, P.O. BOX 80040, 3508-TA, The Netherlands

Maurizio Iaccarino, Professor, Istituto Internazionale di Genetica e Biofisica, via Marconi 12, 80125 Napoli, Italy

Sergei G. Inge-Vechtomov, Department of Genetics and Breeding, St. Petersburg State University, University emb. 7/9, 199034 St. Petersburg, Russia

Andrzej Jerzmanowski, Department of Biology, Warsaw University, 5a Pawinskiego St., 02-106 Warsaw, Poland

Leszek Kaczmarek, Chairman, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteura St., 02-093 Warsaw, Poland

Platon G. Kostyuk, Director, A.A. Bogomoletz Institute of Physiology, 4 Bogomoletz St., 252601 Kiev, Ukraine

Jacek Kuznicki*, Director (from February, 2002), International Institute of Molecular and Cell Biology in Warsaw, Head of Laboratory, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur St., 02-093 Warsaw, Poland

Andrzej B. Legocki, Director, Institute of Bioorganic Chemistry, Polish Academy of Sciences, 12/14 Noskowskiego St., 61-704 Poznan, Poland

Maciej J. Nalecz, Director (till December, 2001), Nencki Institute of Experimental Biology, Polish Academy of Sciences; Chairman (till December, 2001), Polish Network for Molecular and Cell Biology UNESCO/PAN, 3 Pasteura St., 02-093 Warsaw, Poland, Present position: Director of Division of Basic and Engineering Sciences, UNESCO, 1, rue Miollis, 75732 Paris Cedex 15, France

Nathan Nelson, Department of Biochemistry, Faculty of Life Sciences, Tel Aviv University, Ramat-Aviv, Tel Aviv 69978, Israel

Ryszard Przewlocki, 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

Wojciech Stec, Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, 112 Sienkiewicza St., 90-363 Lodz, Poland

Jerzy Vetulani, Deputy Director, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna St., 31-343 Cracow, Poland

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

* 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).

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 |
| January | '01 | Max-Planck Society/Polish Academy of Sciences, Junior Research Group start its activity |
| June | '01 | Professor Jacek Kuznicki elected by International Advisory Board as Director of the Institute. After consultation with UNESCO the official nomination was signed by president of PAN on February 1, 2002. |

Director's note

The third year of formal Institute's activity allowed to reach the phase of certain organisational stability, still leaving enough potential space for expansive growth and development. In 1999 we started with 3 groups working on 9 research topics; currently six research teams of the Institute are in a full swing, focused on approximately 20 research topics. A number of papers accredited to IIMCB exceeded forty. Financing is mainly through the state's budget but extramural funding is significant: nine regular grants from KBN, two KBN's ordered grants, funds from Max Planck Society, Phare Sci-Tech II Project, University of Utrecht, UNESCO and two 5th Framework Projects (one being coordinated at IIMCB). There is a whole list of new grant applications submitted with three extensions to the ongoing 5th FP projects, an application for Center of Excellence status and an application to the Wellcome Trust. IIMCB is active in education with handful of PhD students working on their theses, participation in the exchange with the University of Utrecht, hosting major events of the Postgraduate School of Molecular Medicine and various courses on molecular biology techniques. Next competition for a new lab leader position was advertised. Within last year IIMCB co-organised two international conferences. We look to the future with both great enthusiasm and cautious optimism: a special scientific climate of IIMCB and a quality of research run by our faculty members still overweight financial and organisational problems of everyday life of the Institute.

Andrzej Kozłowski



Jacek Kuznicki

Michal Witt

Descriptions of the Institute's activities in 2001

Organization of research at the Institute. The scope of research carried out in the International Institute is mainly focused on basic biomedical problems. The research work is currently performed in 6 groups: Department of Molecular Biology, Laboratory of Molecular Immunology, Laboratory of Bioinformatics, Laboratory of Molecular Neurology, Laboratory of Structural Biology and Laboratory of Neurodegeneration. Among the major research topics are:

- ♦ the analysis of interactions between human p53 and cytosolic molecular chaperones; Hsp70 as a vehicle for protein therapy; the cloning and characterisation of a novel human protein kinase; the search for new Hsp40 homologues (group of Prof. Zylicz);
- ♦ mast cell mediated modulation of immune response by immunotoxic agents; the search for molecular targets of immunotoxic compounds; development of "fluorescent cell chip" technology for immunotoxicity testing (group of Dr. Dastyh);
- ♦ engineering of the metabolism of fatty acids in flax to produce branched-chain fatty acids with potent biolubricant properties, engineering of DNA methyltransferases, evolution of RNA methyltransferases; (group of Dr. Rychlewski);
- ♦ identification of molecular mechanisms controlling neuronal apoptosis (group of Dr. Hetman);
- ♦ structures of enzymes that are involved in protein degradation, with a particular eye on enzymes that have medical relevance and could be potential drug targets; work on bacterial virulence factors (group of Dr. Bochtler);
- ♦ the search for a functional bio-marker of familial Alzheimer disease (FAD); analysis of calmyrin and its interaction with presenilins; molecular characterization of Polish patients with FAD, sporadic Alzheimer's disease (SAD), and frontotemporal-dementia (FTD); studies of environmental and genetic aspects of longevity (group of Prof. Kuznicki).

Education has been carried out through our own PhD programme (5 students), in collaboration with the Utrecht University, 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 graduated in 2001), with Foundation for Polish Science (2 students), (see section "Educative activities", PhD Programme, p. 36), and by organization of scientific meetings and courses (3 PhD and 5 MSc).

Implementation of research results. Basic research is the core activity of the Institute, however, the possible applications of the research is considered and, if possible, developed. Research is concentrated on a better understanding of the role of various proteins in oncogenesis and on a new generation of prospective anti-cancer compounds. Analysis of the interactions of cellular chaperones and their application as vehicles for protein therapy will be the major issues of this topic. Neurodegenerative disease testing would supplement diagnostic tools in neurology, with special emphasis on the search for a functional bio-marker of FAD. An analysis of healthy ageing will give a bigger picture of this process to gerontologists focusing on collecting information on the environmental, molecular, genetic and social determinants of the process. Work on neuronal

death processes will contribute to an understanding of such neurological disorders as stroke or Parkinson disease and of the deleterious effects of various neurotoxins. All the above-mentioned problems will be analysed in close co-operation with clinical end-users all over the country. The work performed in the crystallographic laboratory also has a potential clinical applicative value.

Popularization of science The Institute actively participates in initiatives aimed at the popularisation of science in Polish society. One example is the Warsaw Science Festival to which the Institute contributes by organising, on its premises, panel discussions, presentations and open laboratory days. In 2001, The Department of Molecular Biology of IIMCB organized an event entitled "Examine your own DNA": a two-day workshop/demonstration with active participation of the audience. The faculty members regularly take part in radio and TV programs and publish articles popularising science. The Institute is frequently presented in the media and is often visited by delegations of foreign journalists, science managers and ministers.

Editing NEWSKO, an electronic bulletin on scientific events on the Ochota Campus (<http://www.iimcb.gov.pl/newsko/newsko.htm>). The Institute puts an emphasis on a constant flow of information and co-operation in the scientific community. "NEWSKO" that has been edited at the Institute for the last four years, integrating scientists, students and medical doctors. This weekly bulletin informs the community about seminars, symposia, conferences and job opportunities. 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. It is also a platform for official announcements about tenders. The current number of subscribers is 110.

The Ochota Campus Neurobiology Club organises a series of seminars on neurobiology at the IIMCB that are open to the scientific community. They are especially addressed to junior scientists working on the Ochota Campus. The main goals of the Club are (i) education in neurobiology and (ii) promotion of scientific interactions

Organization of symposiums and conferences

- ◆ IIMCB and MCBN/UNESCO Symposium "New frontiers in cell and molecular biology -II", Warsaw, June 11, 2001
- ◆ Participation in organization of "The Second International Conference on Inhibitors of Protein Kinases" Warsaw, September 9-15, 2001 (Head of the organizing Committee Prof. D. Shugar, organizers from IIMCB: J. Kuznicki, M. Mossakowska)
- ◆ Participation in organization of the inauguration and review session of students of the Postgraduate School of Molecular Medicine (SMM), Medical University of Warsaw, October 19-20, 2001, Warsaw
- ◆ Co-organizing Workshop "Expression profiling using Affymetrix technology: T helper cell expression profiling using gene chip". Warsaw, November 21, 2001 (organizers: Affymetrix, TK Biotech and IIMCB).

Grants, donations

Grants carried on in 2001

International

- ♦ **5th Framework Programme** (QLK4/CT/2000/00787) "A New Technology for Fluorescent "Cell Chip" Immunotoxicity Testing"; 231,703 EUR (EU) + 207,000 EUR (KBN), 2001-2003, (J. Dastyh)
- ♦ **5th Framework Programme** (QLK3/CT/2000/00349) "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**; 240,000 DM annually from 2001 to 2006 (M. Bochtler)
- ♦ **Utrecht University** fellowships for three PhD students (in M. Zylicz and J. Dastyh labs at IIMCB, and one in Cracow in R. Przewlocki's lab); 55,000 Hfl annually from 2000 to 2003
- ♦ 10 months fellowship for Ms. Sanne Mikkelsen, MSc from the **Polish Academy of Sciences** and the **Danish Academy of Sciences** to perform studies at J. Kuznicki's laboratory at IIMCB

Polish

- ♦ Research 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)
- ♦ Research Grant from Foundation for Polish Science (FNP 15/2000) "Molecular chaperones in cancer cells"; 225,000 PLN for 3 years (2000-2002), (M. Zylicz)
- ♦ Research 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. Dastyh)
- ♦ 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)
- ♦ Research Grant from the State Committee for Scientific Research (KBN) "Engineering of DNA methyltransferases"; 200,000 PLN, (2000-2002), (coordinator – M. Radlinska, co-operators: L. Rychlewski, J. Bujnicki), coordinated at the Biology Department of UW

Grants received in 2001

Polish:

- ♦ Research Grant from the State Committee for Scientific Research (KBN-002\CD\P05\2000) "Molecular and genetic mechanisms of neurodegeneration and neuroprotection"; 260,000 PLN; 2001-2002, within an ordered grant co-ordinated by B. Kaminska-Kaczmarek at the Nencki Institute (M. Hetman)
- ♦ Research Grant from the State Committee for Scientific Research (KBN-002\CD\P05\2000) "Molecular and genetic mechanisms of neurodegeneration and neuroprotection"; 300,000 PLN; 2001-2002, within an ordered grant co-ordinated by B. Kaminska-Kaczmarek at the Nencki Institute (J. Kuznicki)
- ♦ Research Grant from the State Committee for Scientific Research (KBN 0253/P04/2001/21) "Role of glycogen synthase kinase 3 beta in neuronal death"; 270,000 PLN; 2001-2003, (M. Hetman)
- ♦ Research Grant from the State Committee for Scientific Research (KBN 0436/P04/2001\20) "Calcium binding proteins interaction with presenilin 1 (PS1) in lymphocytes of Alzheimer's disease patients and healthy controls"; 325,000 PLN; 2001-2004, (J. Kuznicki)
- ♦ Ordered Grant from the State Committee for Scientific Research (KBN/022/P05/1999) "Genetic and environmental longevity factors in a group of Polish centenarians"; 1,500,000 PLN; 2001-2003, (co-ordinator head J. Kuznicki)
- ♦ Ordered Grant from the State Committee for Scientific Research (PBZ/KBN/033/P05/2000) "Addiction: neurobiological basis, mechanisms, methods of prophylaxis and treatment"; 2001-2003, (co-ordinator head R. Przewlocki)

Support received in 2002

- o Support for the UNESCO Professorship Chair at the IIMCB (ROSTE875.776.1 and 875.777.1); 30,000 USD, (M. Zylicz)

Grants submitted in 2001/2002

- ♦ Research Grant from the State Committee for Scientific Research (KBN) "Hsp90 in cancerogenesis"; 462 000 PLN (A. Wawrzynow), accepted in 2002
- ♦ 5th Framework Programme "Novel non-antibiotic treatment of staphylococcal diseases"; 281,760 EUR, (M. Bochtler)
- ♦ 5th Framework Programme "Exploiting the HSP70 chaperone machine for novel therapeutic strategies in human diseases and for the engineering of productive cellular biomolecular factories"; 130,600 EUR, pending, (M. Zylicz)
- ♦ 5th Framework Programme "Early diagnosis of Alzheimer disease and related dementia. The Cell Factory"; 220,000 EUR, pending (J. Kuznicki)
- ♦ Grant of Wellcome Trust, International Senior Research Fellowship in Biomedical Science in Central Europe, "Activation of Extracellular Signal Regulated Kinases 1/2 as anti-stress response in neurons"; 1,300,000 PLN, (M. Hetman)
- ♦ 5th Framework Programme "Centre of Excellence in Molecular Bio-Medicine"; 529,800 EUR, Application for the Institute.

International contacts

With Max Planck Society

The best example of extremely successful co-operation with the most prestigious European scientific institutions is the agreement between the Max Planck Society (MPG) and Polish Academy of Sciences, which resulted in the creation of a junior research group at IIMCB. The agreement was signed by Prof. M. Mossakowski, President of PAN and Prof. H. Markl, President of MPG in 2001. MPG and IIMCB jointly opened an international competition for a junior research group leader, won by a young German crystallographer, Dr. Matthias Bochtler. In the fall of 2001, the Laboratory of Structural Biology was furnished with the modern equipment and became the most advanced unit of this type not only in Poland but also in Eastern and Central Europe. The laboratory actively co-operates with two twin German Max-Planck Institutes: Institute of Molecular Cell Biology and Genetics in Dresden and Institute of Biochemistry in Martinsried/Planegg. Other active collaborators include: the German biotech company 4SC AG in Munich, Institute of Biotechnology, University in Vilnius, Lithuania and the Institute of Molecular Biology, Jagiellonian University, Cracow, Poland.

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. 32.

With UNESCO

IIMCB and MCBN/UNESCO organized II Symposium "*New frontiers in cell and molecular biology*". Warsaw, June 11, 2001. The programme has been composed of the following sessions: Proteins: Structure and Function (speakers: J. Kuznicki, N. Nelson, M. Zylicz), EMBO Young Investigator Lecture (speaker: I. Konieczny), Signal Transduction – From Membrane to Cell Function (speakers: W. Gispen, M. Hetman, J. Dastyk), Stem Cells (speakers: J-M. Matter, K. Arai, S. Watanabe, Z. Pojda, W. Sawicki, K. Domanska-Janik).

Department of Molecular Biology

Staff:

Maciej Zylicz, PhD (Professor, Head), Alicja Wawrzynow, PhD (Associate Professor), Lech Trzeciak, PhD, Frank King, PhD, Marta Bucko, MSc, Aleksandra Helwak, MSc, Grzegorz Kudla, MSc, Leszek Lipinski, MSc, Malgorzata Gutkowska, MSc, Natalia Kunowska, MSc, Grazyna Orleanska, MSc (secretary), Wanda Gocal (technician)

MACIEJ ZYLICZ, PhD

Degrees:

MSc in physics and biology, University of Gdansk, 1977

PhD in biochemistry, Medical Academy, Gdansk, 1979

DSc in molecular biology, Institute of Biochemistry & Biophysics, PAN, Warsaw, 1986

Professor, 1991

Postdoctoral training:

1979-1981, University of Gdansk, Department of Biochemistry, Gdansk, Poland

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

1993-1994, Visiting Professor, University of Utah, Medical Center, Oncology, USA

Professional employment:

1981-1988, Assistance Professor, Department of Biochemistry, University of Gdansk, Poland

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

1990-1993, Vice President, University of Gdansk

1991-1994, Head of Department of Molecular Biology, University of Gdansk

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

Other professional activities:

2000-2004, Chairman of Biology, Earth Sciences and Environmental Protection Commission of KBN

Membership in scientific societies, organisations and panels:

- ♦ Member of EMBO
- ♦ Member of EMBO Report Editorial Board
- ♦ Member of EMBC
- ♦ Member of Selection Committee for EMBO YIP
- ♦ Member of Selection Committee for the special DFG programmes
- ♦ Member of Polish Academy of Sciences
- ♦ Member of American Society of Biochemistry and Molecular Biology
- ♦ Member of International Advisory Board of IIMCB

- ♦ Member of Academia European
- ♦ Member of the State Committee for Scientific Research
- ♦ Member of Polish Academy of Arts and Sciences

Honours, prizes, awards:

- ♦ Individual Award of Polish Academy of Sciences for scientific achievements, 1986
- ♦ Award of Polish Academy of Sciences, 1990
- ♦ President of Gdansk "Heweliusz" Award for scientific achievements, 1993
- ♦ Award of Ministry of Education, 1994
- ♦ Award of the Polish Biochemical Society for the best biochemistry work performed in Polish laboratories, 1996
- ♦ Award of Foundation for Polish Science, 1999
- ♦ L. Marchlewski Award of Biochemistry and Biophysics Committee PAN, 2001

Publications: circa 70 publications in primary scientific journals

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.* 276: 18843-18848;
- *Genevaux P, Wawrzynow A, Zylicz M, Georgopoulos C, Kelley WL (2001) 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.* 276: 7906-7912;
- *Zylicz M, King F, Wawrzynow A (2001) Hsp70 interactions with the p53 tumour suppressor protein. *EMBO J.*, 20: 4634-4638;
- *King F.W, Wawrzynow A, Hohfeld J, Zylicz M (2001) Cochaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild type or mutant p53. *EMBO J.*, 20: 6297-6305;
- *Zylicz M, Wawrzynow A (2001) Insights into the function of Hsp70 chaperones. *IUBMB*, 51: 283-287;
- *Jassem E, Niklinski J, Rosell R, Niklinska W, Jakobkiewicz J, Monzo M, Chyczewski L, Kobierska G, Skokowski J, Zylicz M, Jassem J (2001) Types and localisation of p53 gene mutations. A report on 332 non-small cell lung cancer patients. *Lung Cancer*, 34: 47-51;
- *Kaczanowski R, Trzeciak L, Kucharczyk K (2001) Multitemperature single-strand conformation polymorphism. *Electrophoresis* 22: 3539-3545;
- Gonciarz-Swiatek M, Wawrzynow A, Um S-J, Learn B.A, McMacken R, Kelly W.L, Georgopoulos C, Sliemers 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, Blaszczyk 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 λ O from RNA transcription-dependent proteolysis by ClpP/ClpX. *Proc. Natl. Acad. Sci. USA* 95, 15259-15263;

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

- 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.

Description of present research:

Analysis of interactions between human p53 and cytosolic molecular chaperones

(F. King, A. Helwak, M. Gutkowska and N. Kunowska, 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. Using highly purified proteins, we have identified intermediate reactions that lead to the assembly of molecular chaperone complexes with wild type or mutant p53R175H protein. Hsp90 possesses higher affinity towards wild type p53 than to the conformational mutant p53R175H. The presence of Hsp90 in a complex with wild type p53 inhibits the binding of Hsp40 and Hsc70 to wild type p53; consequently, preventing the formation of wild type p53-multiple chaperone complexes. The conformational mutant p53R175H can form a stable heterocomplex with Hsp90 only in the presence of Hsc70, Hsp40, Hop and ATP. The anti-apoptotic factor Bag-1 can dissociate Hsp90 from a pre-assembled complex wild type p53 protein, but it cannot dissociate a preassembled p53R175H-Hsp40-Hsc70-Hop-Hsp90 heterocomplex. The results presented in this paper provide possible molecular mechanisms that can help to explain the observed *in vivo* role of molecular chaperones in the stabilization and cellular localization of wild type and mutant p53 protein. 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, M. Zylicz, group leader A. Wawrzynow)

It has been shown recently that the p50 subunit of NF- κ B 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 aim of this study is to elucidate the structure and get some insights into the function of the novel human serine/protein kinase, identified as a fragment of cDNA cloned with degenerated primers-based PCR technique. Full-length cloning of the kinase was based on the RACE technique; during these studies the mouse orthologue was cloned by a Swiss group and termed TSSK3, since it was a third member of a subfamily of protein kinases, expressed in testis Leyding cells. Using HUGO database searches we identified the hypothetical hTSSK3 gene (h standing for human) located on chromosome 1. We designed appropriate primers to human and mouse cDNA and then amplified, cloned and sequenced the genes and the cDNAs from mouse and human. TSSK3 has a very simple structure consisting exclusively of a catalytic domain, with multiple phosphorylation sites including a possible autoactivation loop. We attempted to produce recombinant TSSK3 and experienced a very high frequency of plasmid rearrangements, loss of insert or point mutations in constructs. It was not possible to obtain TSSK3 in a fusion with intein. The fusions with His tag were completely relegated to inclusion bodies (regardless of the induction level or conditions). Placing the construct under tightly-regulated arabinose promoter and varying the expression levels and conditions did not improve the solubility of the protein or stability of the construct. The fusions with GST allowed to obtain a soluble protein. After brief purification of GST fusion we showed that the protein possesses a protein kinase activity against a range of simple substrates (MBP, casein, histone H1) and against itself (autophosphorylation) and also against a yet-unidentified bacterial protein. The kinase is more active in 30°C than in 37°C and strongly prefers Mn⁺⁺ over Mg⁺⁺. We have analyzed the structure of catalytic domain and are now trying to produce kinase-dead and non-activable point mutants, and also fusions to FLAG and HA epitopes under mammalian promoter systems. We have also obtained two immortalized Leydig cell lines that we plan to utilize and are now corroborating the transfection techniques. A batch of the protein has also been chromatographically purified to homogeneity for immunization purposes.

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 autoimmune diseases. We plan to purify new Hsp40 homologues from *E.*

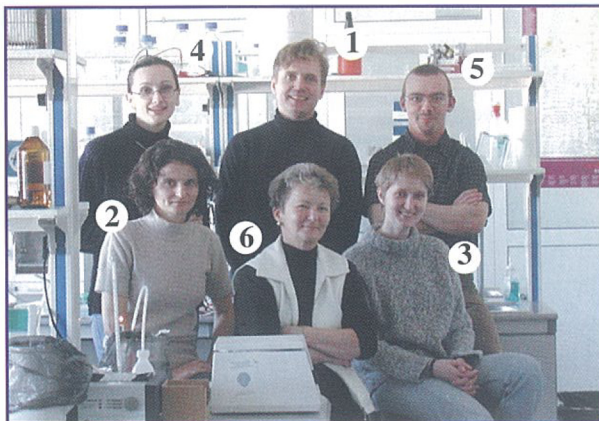
coli and compare their chaperone activity with other, previously identified, Hsp's. This project could potentially lead to identification of a new family of eukaryotic genes, whose protein products are regulated by Hsp70 chaperone.

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 ATPANe 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:

1. Jaroslaw Dastyh, PhD (Head)
2. Urszula Bialek-Wyrzykowska, PhD
3. Dominika Trzaska, MSc
4. Violetta Adamczewska, MSc
5. Maciej Olszewski, MSc
6. Wanda Gocal (technician)



JAROSLAW DASTYCH, PhD

Degrees:

MSc in molecular biology, University of Lodz, Poland 1983
PhD in medical biology, Medical Academy, Lodz, Poland 1991
Habilitation process is pending, Medical Academy, Lodz

Postdoctoral training:

1992-1995, Laboratory of Allergic Diseases, National Institutes of Health, Bethesda, USA

Professional employment:

1983-1985, Technician, Allergy Research Section, Department of Biogenic Amines Polish Academy of Sciences (PAN), Lodz, Poland
1985-1992, Assistant, Allergy Research Section, Department of Biogenic Amines PAN
1992-1995, Senior Researcher, Allergy Research Section, Department of Biogenic Amines PAN
1995-1998, Acting Head, Allergy Research Section, Department of Biogenic Amines PAN

Other professional activities:

1998-1999, Visiting Scientist, Laboratory of Allergic Diseases, National Institutes of Health, Bethesda, USA

Membership in scientific societies, organisations and panels:

Polish Society for Experimental and Clinical Immunology
American Academy of Asthma Allergy and Clinical Immunology

Honours, prizes, awards:

Fulbright Scholarship, 1989
Fogarty International Fellowship, 1992-1995

Publications: **circa 20 publications in primary scientific journals**

Selected publications since 1997:

- *Taylor M, Dastyh J, Sehgal D, Sundstrom M, Nilsson G, Akin C, Mage RG, Metcalfe DD (2001) The kit activating mutation D816V enhances stem cell factor-dependent chemotaxis. *Blood*. 98: 1195-1199;
- Dastyh J, Wyczolkowska J, Metcalfe DD (2001) Characterization of $\alpha 5$ -integrin-dependent mast cell adhesion following Fc ϵ RI aggregation. *Int. Arch. Allergy Immunol.* 125: 152-159;
- Wyczolkowska J, Weyer A, Dastyh J (2000) Inhibitory effect of wheat germ agglutinin on mouse mast cell adhesion to fibronectin. *Int. Arch. Allergy Immunol.* 122: 216-223;
- *Fukui M, Whittlesey K, Metcalfe DD, Dastyh J (2000) Human mast cells express the hyaluronic-acid-binding isoform of CD44 and adhere to hyaluronic acid. *Clin. Immunol.* 94: 173-178;
- Dastyh J, Walczak-Drzewiecka A, Wyczolkowska J, Metcalfe DD (1999) Murine mast cells exposed to mercuric chloride release granule associated N-acetyl-b-D-hexosaminidase and secrete IL-4 and TNF- α . *J. Allergy Clin. Immunol.* 103: 1108-1114;
- Dastyh J, Taub D, Hardison MC, Metcalfe DD (1998) Tyrosine kinase deficient Wv c-kit induces mast cell adhesion and chemotaxis. *Am. J. Physiol.* 44: C1291-C1299;
- Dastyh J, Hardison MC, Metcalfe DD (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, Dastyh J, Metcalfe DD, Mekori YA (1997) Extracellular matrix-anchored serum amyloid A preferentially induces mast cell adhesion. *Am. J. Physiol.* 273 (1 Pt 1): C179-C187;
- Mekori YA, Oh CK, Dastyh J, Goff JP, Adachi S, Bianchine PJ, Worobec A, Semere T, Pierce JH, Metcalfe DD (1997) Characterization of a mast cell line that lacks the extracellular domain of membrane c-kit. *Immunology* 90: 518-525.

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

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 dysregulation and to abnormal immune response that might be harmful to the organism either by an inadequate and inefficient defense against parasites or neoplasms 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, HgCl₂-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 HgCl₂-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:

Leszek Rychlewski, MD, PhD (Head), Janusz Bujnicki, PhD

LESZEK RYCHLEWSKI, MD, PhD

Degrees:

MSc in medicine, Medical Faculty, Charite, Humboldt University in Berlin, Germany, 1996
MD in medicine, degree at the Medical Faculty, Charite, Humboldt University in Berlin under the supervision of Prof. Cornelius Froemmel, 1998

Postdoctoral training:

1998-1999, San Diego Supercomputer Center, Computational Biology Center, USA

Professional employment:

1996-1998, Research fellow, Scripps Research Institute, Department of Molecular Biology, La Jolla, USA

Other professional activities:

1988-1996, Student at the Humboldt University in Berlin, Germany

Publications: **27 publications in primary scientific journals (1999-2001)**

Selected publications since 1999:

- *Bujnicki JM, Radlinska M, Rychlewski L (2001) Polyphyletic evolution of type II restriction enzymes revisited: two independent sources of second-hand folds revealed. *Trends Biochem. Sci.* 26: 9-11;
- *Pawlowski K, Rychlewski L, Zhang B, Godzik A (2001) Fold predictions for bacterial genomes. *J. Struct. Biol.* 134: 219-231;
- *Bujnicki JM, Rychlewski L (2001) Unusual evolutionary history of the tRNA splicing endonuclease EndA: relationship to the LAGLIDADG and PD-(D/E)XK deoxyribonucleases. *Protein Sci.* 10: 656-660;
- *Lundstrom J, Rychlewski L, Bujnicki JM, Elofsson A (2001) Pcons: a neural-network-based consensus predictor that improves fold recognition. *Protein Sci.* 10: 2354-2362;
- *Bujnicki JM, Elofsson A, Fischer D, Rychlewski L (2001) LiveBench-1: continuous benchmarking of protein structure prediction servers. *Protein Sci.* 10: 352-361;
- *Bujnicki JM (2001) In silico analysis of the tRNA: mIA58 methyltransferase family: homology-based fold prediction and identification of new members from Eubacteria and Archaea. *FEBS Lett.* 507: 123-127;
- *Bujnicki JM, Elofsson A, Fischer D, Rychlewski L (2001) Structure prediction meta server. *Bioinformatics* 17: 750-751;

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

Rychlewski L (2001) ToolShop: prerelease inspections for protein structure prediction servers. *Bioinformatics* 17: 1240-1241;

- *Bujnicki JM, Rotkiewicz P, Kolinski A, Rychlewski L (2001) Three-dimensional modeling of the I-TevI homing endonuclease catalytic domain, a GIY-YIG superfamily member, using NMR restraints and Monte Carlo dynamics. *Protein Eng.* 14: 717-721;
- *Bujnicki JM, Rychlewski L (2001) Reassignment of specificities of two cap methyltransferase domains in the reovirus lambda2 protein. *Genome Biol.* 2: 38;
- *Bujnicki JM, 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* 267: 183-191;
- *Bujnicki JM, 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* 22: 219-230;
- *Bujnicki JM, 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.* 3: 69-72;
- *Bujnicki JM, Feder M, Radlinska M, Rychlewski L (2001) mRNA: guanine-N7 cap methyltransferases: identification of novel members of the family, evolutionary analysis, homology modeling, and analysis of sequence-structure-function relationships. *BMC Bioinformatics* 2: 2;
- *Cristobal S, Zemla A, Fischer D, Rychlewski L, Elofsson A (2001) A study of quality measures for protein threading models. *BMC Bioinformatics* 2: 5;
- *Bujnicki JM, Rychlewski L (2001) Sequence analysis and structure prediction of aminoglycoside-resistance 16S rRNA:m7G methyltransferases. *Acta Microbiol. Pol.* 50: 7-17;
- *Radlinska M, Bujnicki JM (2001) Site-directed mutagenesis defines the catalytic aspartate in the active site of the atypical DNA: m4C methyltransferase M.NgoMXV. *Acta Microbiol. Pol.* 50: 93-101;
- *Radlinska M, Bujnicki JM (2001) Cloning of enterohemorrhagic *Escherichia coli* phage VT-2 dam methyltransferase. *Acta Microbiol. Pol.* 50: 157-163;
- *Bujnicki JM, Radlinska M (2001) Cloning and characterization of M.LmoA118I, a novel DNA: m4C methyltransferase from the *Listeria monocytogenes* phage A118, a close homolog of M.NgoMXV. *Acta Microbiol. Pol.* 50: 155-166;
- *Bujnicki JM (2001) Understanding the evolution of restriction-modification systems: clues from sequence and structure comparisons. *Acta Biochim. Pol.* 48: 935-967;
- *Bujnicki JM, Radlinska M, Zaleski P, Piekarczyk A (2001) Cloning of the *Haemophilus influenzae* Dam methyltransferase and analysis of its relationship to the Dam methyltransferase encoded by the HP1 phage. *Acta Biochim. Pol.* 48: 969-983;
- *Bujnicki JM (2001) A model of structure and action of Sau3AI restriction endonuclease that comprises two MutH-like endonuclease domains within a single polypeptide *Acta Microbiol. Pol.* 50: 219-229;
- *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 Lett.* 486: 328-329;

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

- *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, Elofsson 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 JC, 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 JM (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 JM (2000) Molecular modeling of M.BssHII methyltransferase. Is circular permutation common to all DNA methyltransferase subfamilies? *Int. J. Biol. Macromol.* 27: 195-204;
- *Bujnicki JM (2000) Sequence, structural and evolutionary analysis of prokaryotic ribosomal protein L11 methyltransferases. *Acta Microbiol. Pol.* 49: 19-29;
- *Bujnicki JM (2000) Phylogeny of the restriction endonuclease-like superfamily inferred from comparison of protein structures. *J. Mol. Evol.* 50: 39-44;
- 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 JS, 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 JM., Radlinska M (1999) Molecular phylogenetics of DNA 5mC-methyltransferases. *Microbiol. Pol.* 48: 19-33;
- Bujnicki JM., 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 JM (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 JM., 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 JM (1999) Cloning of the Dam methyltransferase gene from *Haemophilus influenzae* bacteriophage HP1. *Microbiol. Pol.* 48: 123-129;
- Kelley LA, MacCallum RM, 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.

Projects in 2001:

- ♦ "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 from 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. **J. Bujnicki** (P.I.).

Laboratory of Molecular Neurology

Staff:

1. Michal Hetman, MD, PhD (Head)
2. Agata Gozdz, MSc
3. Agata Habas, MSc
4. Magdalena Kuzma, MD
(co-worker from Medical
University of Warsaw)



MICHAL HETMAN, MD, PhD

Degrees:

MD, Medical Academy, Warsaw, Poland, 1994

PhD in neurobiology, Center of Experimental and Clinical Medicine, PAN, Warsaw, Poland, 1997

Postdoctoral training:

1997-2000, Drs. Zhengui Xia and Daniel R. Storm, Departments of Pharmacology and Environmental Health, University of Washington, Seattle, USA

Professional activities:

1989-1991, trainee at the Department of Histology and Embryology, Warsaw Medical School, Poland

1991-1992, trainee at the Department of Clinical Cytology, Centre of Postgraduate Medical Education, Warsaw, Poland

1995-1996, FEBS fellowship to support a project "Role of Cathepsin D in neuronal death", Zentrum Biochemie, Universitaet Goettingen, Germany

1996, summer internship, laboratory of Dr. Mariano Barbacid, Bristol-Myers-Squibb Pharmaceutical Research Institute, Princeton, USA

1996, INSERM fellowship to support a project "Regulation of Cathepsin D expression in neurotoxic damage of rat brain", INSERM Unite 148, Montpellier, France

Professional employment:

1991-1992, PhD studies, Abteilung Biochemie 2, Zentrum Biochemie, Universitaet Goettingen, Germany

1993-1997, PhD student, The Nencki Institute of Experimental Biology, Warsaw, Poland

1994-1995, intern in CSK WAM University Hospital, Warsaw, Poland

1997-2000, Postdoctoral Fellow, Department of Pharmacology, University of Washington, USA

1995-2000, Senior Research Associate, The Nencki Institute of Experimental Biology PAN

2000-present, PhD Scientist in Laboratory of Molecular Neurobiology, The Nencki Institute of Experimental Biology PAN

Membership in scientific societies, organisations and panels:

Member of Polish Biochemical Society

Honours, prizes, awards:

Young Scientist Award of the Foundation for Polish Science, 1995

Polish Prime-Minister prize for distinctive doctoral thesis, 1998

Two-year postdoctoral fellowship award of American Heart Association, 1998-2000

One-year postdoctoral fellowship award of American Heart Association, 2000

Publications: 19 publications in primary scientific journals

Selected publications since 1997:

*Habas A, Gozdz A, Hetman M (2001) Apoptosis as a causative factor and a therapeutic opportunity in pathologies. *Ann. Diagn. Ped. Pathol.* 5: 19-25;

Figueroa-Masot XA, Hetman M, Higgins M, Kokot N, Xia Z (2001) Taxol Induces Apoptosis In Cortical Neurons by a Mechanism Independent Of Bcl-2 Phosphorylation *J. Neurosci.* 21: 4657-67;

Wong ST, Baker LP, Trinh K, Hetman M, Suzuki LA, Storm DR, Bornfeld KE (2001) Adenylyl cyclase 3 mediates prostaglandin E2-induced growth inhibition in arterial smooth muscle cells. *J. Biol. Chem.* 276: 32046-53;

Cavanaugh JE, 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.* 21: 434-443;

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 RJ, Morrison RS (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 JE, 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 J, Biedermann IW, Lapinska J, Szklarczyk A, Figiel I, Konopka D, Nowicka D, Filipkowski RK, 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 AW, Filipkowski RK, Trauzold A, Nowicka D, Hetman M, Kaczmarek L *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;

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

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:

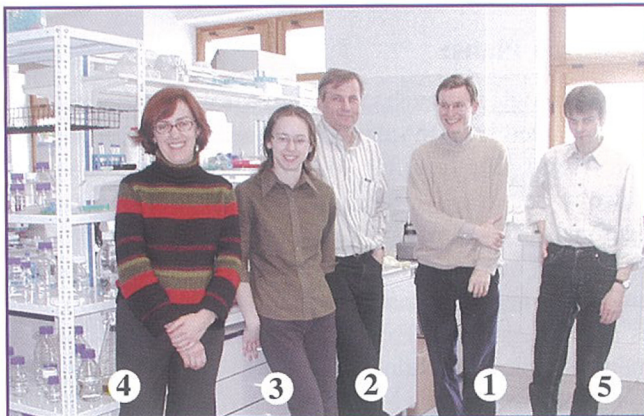
Neurological diseases including stroke, Alzheimer's and Parkinson's pathologies are accompanied by DNA damage followed by neuronal death in the central nervous system (CNS). The resulting loss of nerve cells is thought to underlie neurological dysfunction occurring in these conditions. Therefore therapeutic intervention to enhance neuronal survival may prevent development of deleterious neurological symptoms. The research of our laboratory is concentrated on identification of molecules controlling the process of neuronal death. Currently we study the involvement of two signaling kinases in this regulation. Extracellular signal Regulated Kinase 1/2 (ERK1/2) is a key element of a signal transduction pathway regulating proliferation, differentiation and neuronal survival. We have found that activation of ERK1/2 protects cultured cortical neurons from apoptosis induced by DNA damage. Interestingly, we have also revealed ERK1/2 activation by DNA damage. This activation is protective as ERK1/2 inhibition greatly potentiates DNA damage induced neuronal apoptosis. Currently we investigate the mechanisms of (i) ERK1/2 mediated protection and (ii) ERK1/2 activation in response to genomic injury. Glycogen Synthase Kinase 3beta (GSK3beta) is activated after trophic deprivation and contributes to apoptosis induced by withdrawal of trophic support. GSK3beta is also upregulated in Alzheimer's pathology. Therefore we investigate the possible involvement of GSK3beta in neuronal death triggered by disease-related stimuli such as excitotoxins, hypoxia or oxidants. We also study the regulation of GSK3beta activity in CNS neurons. Interestingly, we have found that proapoptotic activity of GSK3beta is inhibited by ERK1/2 pathway to enhance neuronal survival. In our research we use a multidisciplinary approach combining techniques of cell biology, molecular biology, pharmacology and biochemistry. The main system that is used in the lab are primary cultures of cortical neurons from newborn rats. The culture procedure used in our lab results in more than 80% of cells staining for neuronal marker MAP2 and about 10% of cells expressing astroglia marker, GFAP. Cultured cortical neurons can be studied using both cell biology and biochemical approaches. They can also be manipulated by transfection of expression plasmids.

Laboratory of Structural Biology

(Joint MPG-PAN Junior Research Group)

Staff:

1. Matthias Bochtler, PhD (Head)
2. Roman Szczepanowski, MSc
3. Renata Filipek, MSc
4. Izabela Sabala, MSc
5. Sergey Odintsov, MSc



MATTHIAS BOCHTLER, PhD

Degrees:

MSc in experimental physics, Ludwig Maximilians-Universität München, Germany, 1995
PhD in biochemistry, Technische Universität München, Germany, 1999

Postdoctoral training:

1999-2000, Max-Planck-Institut für Biochemie, Martinsried, Germany

Professional activities:

1990-1992, studies in physics, Munich University, Germany
1992-1993, guest student, Cambridge University, United Kingdom
1995-1996, internship, the Department of Medical Microbiology, University of Regensburg, Germany

Honours, prizes, awards:

Scholarship from Deutsche Studienstiftung and the Bavarian state, 1990-1992
Crystal award, Germany, 1998
Crystal award, Germany, 2000

Publications: 8 publications in primary scientific journals

Selected publications since 1997:

- Bochtler M, Hartmann C, Bourenkov GP, Bartunik HD, 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 DH, 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 HD, 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.

Description of present research:

The laboratory of Structural Biology started its activity officially May 15th, 2001 after a half-year interim period to establish the wet lab and to purchase the crystallographic equipment. The group has since grown to a total of four people, with Roman Szczepanowski, Renata Filipek and Sergey Odintsov starting in spring, summer and autumn, respectively. During the last year, we managed to set up the crystallographic equipment and started actual activities in the lab.

Scientifically, we are interested in structures of enzymes that are involved in protein degradation, with a particular eye on enzymes that have medical relevance and could be potential drug targets. We have started work on two classes of enzymes of the ubiquitin pathway, the ubiquitin activating enzymes (E1s) and the class II ubiquitin hydrolases (class II UCHs). After some initial difficulties, we have now managed to establish a purification protocol for an E1 that gives us sufficient amounts of active protein for crystallisation experiments. Cloning and expression of the ubiquitin hydrolases proved to be difficult, but thanks to the help of Dr. David Drechsel of the Max-Planck-Institute of Molecular Cell Biology and Genetics in Dresden, we now have one working expression system for a class II UCH. Work on inhibitors of the UCHs went more smoothly. Together with Prof. Izdebski, Dept. of Chemistry, Warsaw University, we have designed a peptidomimetic that we expect to act as a UCH inhibitor. The compound, that was synthesized in Prof. Izdebski's lab, is ready, and we hope to test it in the near future, at least in vitro.

The second major activity of the lab has been our work on bacterial virulence factors in collaboration with Prof. Jan Potempa, Krakow University. We managed to express, purify and crystallize prostaphopain, a bacterial proteinase that is suspected to act as a bacterial virulence factor when present in its mature form. We have recently produced the selenomethionine protein and hope to solve the structure with anomalous phasing. As the propeptide acts as an extremely potent inhibitor of the mature form, we hope to learn from this complex for the design of small molecule inhibitors for this protease. A collaboration with a Munich based biotech company has been agreed to exploit the expected information from the crystal structure.

In addition to these core activities of the lab, we have collaborated with a number of Polish groups to solve structures of enzymes that these groups are interested in. To everybody's great disappointment, a well diffracting "ClpXP" crystal grown in collaboration with Dr. Alicja Wawrzynow and Prof. Maciej Zylicz turned out to contain only the structurally known component, ClpP. Currently, we also collaborate with Prof. David Shugar and Dr. Lobočka, both of Warsaw, and Prof. Jozef Kur of Gdansk. Finally, we have been working quite closely with the group of Drs. Virgis Siksnys and Saulius Grazulis of the Institute of Biotechnology in Vilnius, Lithuania.

Laboratory of Neurodegeneration

Staff:

1. Jacek Kuznicki, PhD
(Professor, Head)
2. Urszula Wojda, PhD
3. Cezary Zekanowski, PhD
- 4.. Malgorzata Mossakowska, PhD
(Centenarian Programme)
5. Sanne Mikkelsen, MSc
6. Aleksandra Szybinska, MSc
(Cell and DNA bank in Centenarian Programme),
7. Tomasz Rudka



JACEK KUZNICKI, PhD

Degrees:

MSc in biochemistry, Warsaw University, Poland, 1976

PhD in biochemistry, The Nencki Institute of Experimental Biology PAN, Warsaw, Poland, 1980

DSc The Nencki Institute of Experimental Biology PAN Professor, 1993

Postdoctoral training:

1980-1981, Postdoctoral Fellow, Nencki Institute of Experimental Biology PAN

1981-1984, Postdoctoral Fellow, National Institutes of Health, Bethesda, USA

Professional activities:

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

2000-2001, Director of Center of Excellence for Studies on Mechanisms of Neurodegeneration Phare Sci-Tech II located at the Nencki Institute of Experimental Biology PAN

Professional employment:

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

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

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

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

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

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

1998-present, Acting Director and Head of the Laboratory of Neurodegeneration, IIMCB in Warsaw, Poland

February 2002 – present, Director, International Institute of Molecular and Cell Biology

Membership in scientific societies, organisations and panels:

Member of the Polish Biochemical Society, 1977
General Secretary of the Polish Biochemical Society, 1989-1991
Co-Editor of *Advances in Biochemistry* (published in Polish), 1989-1992
Member of the Polish Biotechnology Committee, 1990
Vice-president of the Polish Biotechnology Committee, 1996-1999 and 2000-2002
Member of the Polish Society for the Advancement of Science and Arts, 1991
Member of the Polish Neuroscience Society, 1991
Member of the Biochemical Society (England), 1995

Honours, prizes, awards:

Diploma cum laude, University of Warsaw, 1976
Mozolowski Award of the Polish Biochemical Society for outstanding Polish young biochemists, 1977
Parnas Award of Polish Biochemical Society for publishing of the best paper in biochemical research, 1977
Skarzynski Award of Polish Biochemical Society to for the best review article in *Advances in Biochemistry*, 1986
Polish Anatomical Society Award for the article on calcium binding proteins published in "Advances in Cell Biology", 1987
Award from Division of Biological Sciences PAN for the work on calcium binding proteins, 2001

Expert evaluation:

2001, ordered opinion for the Polish Academy Sciences concerning regulations for the usage of genetically modified organisms (GMO)
1995, present, standing reviewer of *Biochemical Journal* as the Editorial Advisor

Publications: 57 publications in primary scientific journals

Selected publications since 1997:

- *Filipek A, Jastrzebska B, Nowotny M, Kuznicki J (2002) Siah1 Binding Protein (CacyBP/SIP) interacts with Ca²⁺- binding proteins of the S100 family, *submitted*;
- *Filipek A, Jastrzebska B, Nowotny M, Kwiatkowska K, Hetman M, Surmacz L, Wyroba E, Kuznicki J (2002) Ca²⁺-dependent Translocation of Calcyclin Binding Protein in Neurons and Neuroblastoma NB-2a Cells, *J. Biol. Chem.*, *in press*;
- *Palczewska M, Groves P, Ambrus A, Kaleta A, Kover KE, Batta G, Kuznicki J (2001) Structural and biochemical characterization of neuronal calretinin domain I-II (residues 1-100). Comparison to homologous calbindin D28k domain I-II (residues 1-93). *Eur. J. Biochem.* 268: 6229-6237;
- *Mossakowska M, Puzianowska-Kuznicka M, Barcikowska M, Chiron-Jouan S, Czyzewski K, Derejczyk J, Franceschi C, Gabrylewicz T, Galus K, Grodzicki T, Gross R, Klich-Raczka A, Luczywek E, Passeri G, Pfeffer A, Pruszyński J, Radziszewska E, Sikora E, Sosnowski M, Styczynska M, Wasiak B, Wieczorowska-Tobis K, Zyczkowska J, Kuznicki J (2000)

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

Polish Centenarians Program "PolStu99" – search for longevity promoting factors (in Polish) *Gerontologia* 8: 35-39;

- Nowotny M, Bhattacharya S, Filipek A, Krezel A.M, Chazin W, Kuznicki J (2000) Characterization of the interaction of calyculin (S100A6) and calyculin-binding protein. *J. Biol. Chem.* 275: 31178-31182;
- Jastrzebska B, Filipek A, Nowicka D, Kaczmarek L, Kuznicki J (2000) Calyculin (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 calyculin (S100A6) gene. *Biochim. Biophys. Acta* 1517: 73-81;
- Lesniak W, Swart GWM, Bloemers HPJ, Kuznicki J (2000) Regulation of cell specific expression of calyculin (S100A6) in nerve cells and other tissues. *Acta Neurobiol. Exp.* 60: 569-575;
- Palczewska M, Groves P, Kuznicki J (1999) Use of Pichia PANtoris 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 calyculin. *J. Neurochem.* 70: 1793-1798;
- Groves P, Finn BE, Kuznicki J, Forsen S (1998) A model for target protein binding to calcium-activated S100 dimers. *FEBS Lett.* 421: 175-179;
- Strauss KI, Kuznicki J, Winsky L, Hammer M, Jacobowitz DM (1997) The mouse calretinin gene promoter region: structural and functional components. *Mol. Brain. Res.* 49: 175-187.

Present projects:

The search for a functional bio-marker of familial Alzheimer disease (FAD) - identification of the proteins 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 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. We use immortalized lymphocytes, in which we sequenced of PS1, PS2, APP and identified APOE isoforms.

Analysis of calmyrin and its interaction with presenilins

We cloned human calmyrin (a mirystoylated calcium-binding protein) by RT-PCR into a pET28a vector to construct a His-tagged version of the protein. The protein is used to assess the binding properties of calmyrin to wild type and mutated presenilins by affinity chromatography. It is known that calmyrin binds to a part of the loop region of presenilin 1 and 2, but it is unknown what part of calmyrin binds the loop region. We intend to elucidate the location of the binding region through the use of calmyrin deletion mutants.

Studies of molecular misreading in AD patients

Molecular misreading is a process of inaccurate conversion of genomic information from DNA into frameshift transcripts and truncated proteins (+1 proteins). It is caused mainly by dinucleotide or single nucleotide deletions in, or adjacent to, e.g. a GAGAG motif in mRNA transcripts. Expression of ubiquitin+1, APP+1 and other aberrant transcripts in neuronal tissues could underlie the pathogenesis of sporadic Alzheimer's disease and other neurodegenerative conditions as well.

Molecular misreading can be detected at the protein level, using antibodies against +1 proteins. Recently van den Hurk and colleagues used a bacterial expression system with GFP as a reporter to screen gene transcripts for molecular misreading. However this approach is time-consuming, as cloning and colony screening is needed.

We prepare a simple PCR test using peptide nucleic acids to detect aberrant transcripts and will try to check if molecular misreading is active in various tissues.

Molecular characterization (genotypes at PS1, PS2, APP and MAPT) of Polish patients with familial Alzheimer's disease, sporadic Alzheimer's disease patients (SAD), and frontotemporal-dementia (FTD)

An attempt to identify polymorphisms at the novel intronic gene (Saitohin) will be performed in a group of SAD patients. The aim of the research is to correlate genotypes with particular clinical outcome and found polymorphisms modulating the individual risk for AD and FTD.

Polish Centenarians Programme "Studies on environmental and genetic aspects of longevity" (<http://www.iimcb.gov.pl/centenarians/centenarians.htm>)

This multidisciplinary, 3 years long Programme entitled "Environmental and genetic factors of Polish centenarians' longevity" and funded by KBN has started at the end of 2001. The package is coordinated by J. Kuznicki, the organization of medical examination, blood analysis and database by M. Mossakowska, and it consists of 7 original projects, in which 22 research groups from different labs in Poland take part:

- ♦ 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 Szlag
- ♦ 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 Klapinska
- ♦ Establishment of DNA, RNA and immortalized lymphocytes bank. Study on chromosomal aberrations and polymorphism of genes connected with ageing, Dr. Michal Witt.

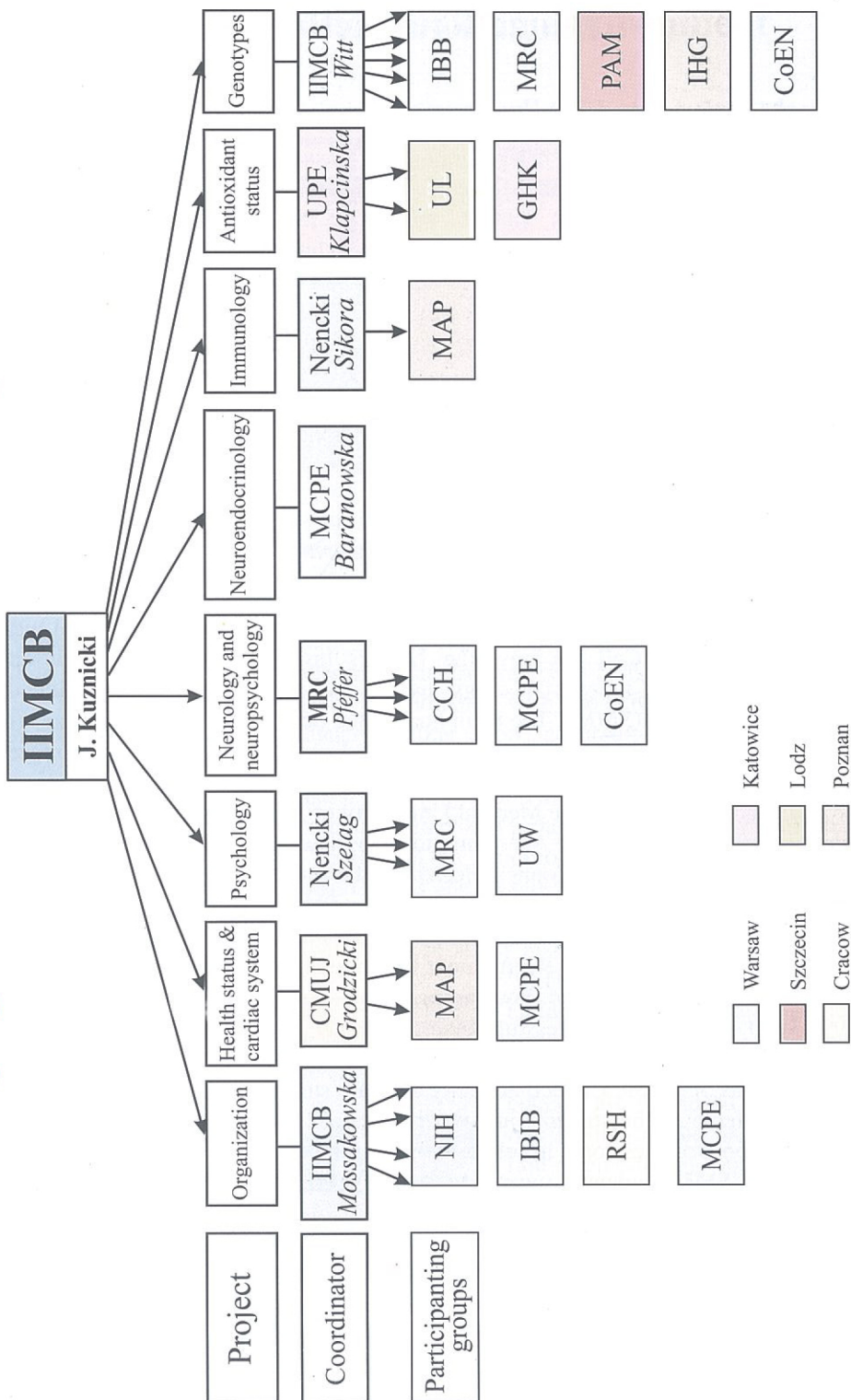
The scientific aim of the Programme is to collect information on the environmental determinants of healthy aging in Polish centenarians and to provide material to study

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 pilot of this Programme started at IIMCB in autumn 1998 and its aims, and data were described in Gerontologia 8 (4) 35-39, 2000.

List of research groups participating in "PolStu2001"

1. **CCH** – Central Clinical Hospital of Ministry of the Interior and Administration, Clinic of Neurology, Warsaw
2. **CoEN** – Centre of Excellence for Studies on Neurodegeneration, Phare Sci-Tech, Warsaw
3. **CMUJ** – Collegium Medicum, Jagiellonian University, Department of Gerontology, Cracow
4. **GHK** – Geriatric Hospital, Katowice
5. **IBB** – Institute of Biochemistry and Biophysics, PAN/University of Warsaw, Department of Genetics
6. **IIMCB** – International Institute of Molecular and Cell Biology in Warsaw
7. **IBIB** – Institute of Biocybernetics and Biomedical Engineering, PAN, Warsaw
8. **IHG** – Institute of Human Genetics, PAN, Poznan
9. **MAP** – Medical Academy, Department of Pathophysiology, Poznan
10. **MCPE** – Medical Centre of Postgraduate Education , Warsaw
 - ♦ Clinic of Familiar Medicine
 - ♦ Clinic of Ophthalmology
 - ♦ Department of Biochemistry
11. **MRC** – Medical Research Centre, PAN, Department of Neurodegenerative Disorders, Warsaw
12. **Nencki** – Nencki Institute of Experimental Biology, PAN, Warsaw
 - ♦ Laboratory of Neuropsychology, Department of Neurophysiology
 - ♦ Department of Cellular Biochemistry
13. **NIH** – National Institute of Hygiene, Warsaw
14. **PAM** – Pomeranian Medical Academy, Department of Clinical Biochemistry and Laboratory Diagnostic, Szczecin
15. **UFE** – University of Physical Education, Katowice
16. **RSH** – Regional Specialistic Hospital of Ludwik Rydygier, Cracow
17. **UL** – University of Lodz, Department of Molecular Biophysics
18. **UW** – University of Warsaw, Faculty of Psychology

Organigramme of the Polish Centenarians Programme



Educational activities

Utrecht University PhD Programme

The Utrecht University PhD 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. 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. The PhD programme itself offers three 4-year doctoral positions (based on financial support of 55.000 Dutch guilders a year). The PhD 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 (www.smm.edu.pl): 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 PhD 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 PhD programme. The candidates are requested to present a scientific programme of their PhD study, scientific merit of which is carefully evaluated by the Recruitment Committee of SMM as well as independent referees in Poland and abroad. Four groups of students were accepted so far: in 1998, 1999 2000 and in 2001. 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.

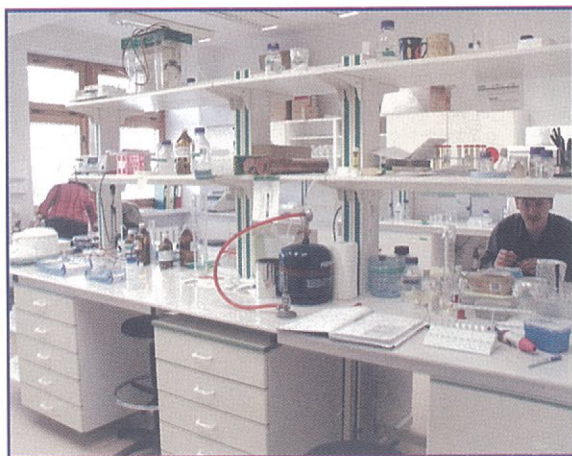
Infrastructure and working environment

The infrastructure of the Institute is fully adapted to the safety and bio-safety regulations for chemistry and molecular biology laboratories. All laboratories have been furnished and contain new and modern pieces of equipment according to the highest international standards. In addition to typical equipment in each wet laboratory, there are pieces of equipment shared by all



researchers. These are centrifuges and ultracentrifuges, sets of FPLC systems, a real-time thermocycler, incubators and shakers for bacterial cultures, electroporator for transfections and transformations, freezers -70°C. There are also five cell culture labs equipped with incubators, laminars, and microscopes, three cold rooms, and two sets of water deionising units. The laboratory for isotopes will be constructed in 2002 with respect to Polish and EU law regulations. The Laboratory of Structural Biology, fully financed by the Max-Planck-Society, is furnished with the most modern research equipment including 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. Because of its uniqueness, this equipment will serve members of the whole scientific community displaying particular interest in protein crystallography analysis.

The building is equipped with ventilation, air conditioning, smoke alarms and fire escapes according to the regulations. Offices and lecture halls are separated from the laboratory space. The infrastructure for clean animals' husbandry is prepared. The equipment for transgenic animals will be successively installed.



Three lecture halls allow for an intensive seminar programme, without unnecessary restrictions on time schedules. The practical courses are organised in a separate laboratory that is an important element for comfort and work safety. Being part of a large building complex, IIMCB has access to: 6 lecture halls from 20 to 300 persons), exhibition hall, hotel, cafeteria as well as to other facilities of neighbour research institutes of the Ochota Campus.

The IIMCB facilities, as well as the whole campus complex, are fully accessible to disabled people. Medical, social and legal services are accessible to the whole staff. A cafeteria offers sandwiches and lunches.

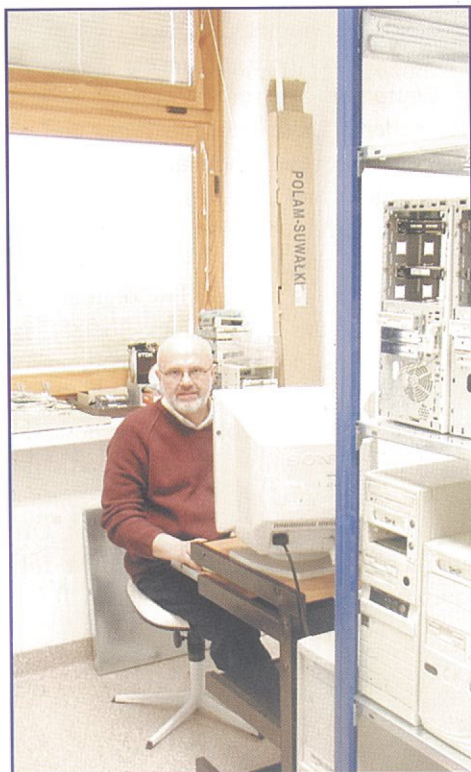
The Institute laboratories and facilities are accessible around the clock since the buildings are under guard-security services.

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 three 3Com 24-ports switches Ethernet 10/100 Mb/s.

There are about 60 workstations, notebooks and pads in the network protected by the local firewall 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 and a cluster of eight 2-processors servers under Linux used for computation. Six printers work as network devices and 2 modems connect computers with the telephone system allowing users remote access from home.

The next year we plan to implement a connection to the rest of the Ochota campus through ATM 622 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.

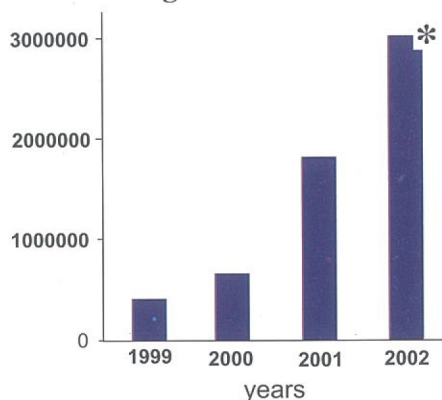
Financial Statements

INCOME and EXPENSES of the INTERNATIONAL INSTITUTE of MOLECULAR and CELL BIOLOGY I - XII 2001

I.	BUILDING EXPLOITATION	405 980,68
1.	Electricity, hot water and other utilities	153 994,20
2.	Conservation, renovation	164 491,78
3.	Cleaning, security	46 724,87
4.	Mail, telephone bills	40 769,83
II.	RESEARCH and OPERATION COSTS	2 325 153,17
1.	Equipment, furniture, computers	141 455,91
2.	Laboratory research equipment	195 019,77
3.	Materials	724 786,30
4.	Amortization	218 872,17
5.	Taxes	187 810,52
6.	Other	857 208,50
III.	SALARIES	1 772 483,42
1.	Salaries	1 443 400,16
2.	Contribution for National Insurance Scheme	241 243,11
3.	Year reward	87 840,15
IV.	TOTAL COSTS	4 503 617,27
1.	BUDGETARY DONATIONS	2 363 900,00
2.	GRANTS – KBN, FNP	2 217 900,00
3.	GRANTS – UNESCO, EU, MAX PLANCK	1 103 434,98
4.	OTHER INCOME	519 240,09
V.	TOTAL INCOME	6 204 475,07
1.	LABORATORY RESEARCH EQUIPMENT – INVESTMENT COSTS	382 210,86

Diversity of funding

Annual budget of the IIMCB and sources of funding



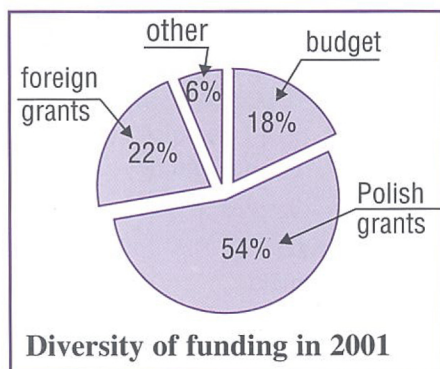
The annual budget of the IIMCB grew from 397 942 to 1 809 593 EURO in the last three years. The expected* budget in 2002 is calculated to be approximately 3 018 400 EURO. An overview of the main lines for 2001 income is given in Table 1.

Source	Budgetary line	Amount in PLN	Amount in EURO*
Budget donation		1 199 000.00	326 703.00
State Committee for Scientific Research (KBN)	Statutory grant	790 000.00	215 258.86
State Committee for Scientific Research (KBN)	Special research programme	374 900.00	102 152.59
State Committee for Scientific Research (KBN)	Heavy equipment investment grant	205 000.00	55 858.31
State Committee for Scientific Research (KBN)	Research grants	2 184 900.00	595 340.60
Other Polish grants	FNP grant	43 800.00	11 934.60
European Union	Grants	609 232.00	166 003.27
Max Planck Society	Grant	651 781.00	177 597.00
Other international grants and projects	Foreign grants	174 396.00	47 519.35
Commercial activities, financial investments, donations		408 200.00	111 226.16
Total		6 641 209.00	1 809 593.74

Table 1. 2001 IIMCB income (1 EURO = 3.67 PLN)

The Institute's budget donation from the central State budget is designed for:

- ♦ maintenance of the building (heat, electricity, security systems)
- ♦ administration



The Institute statutory budget covers:

- ♦ salaries and scholarships for researches (39% of the budget)
- ♦ infrastructure (commodities, small apparatus)
- ♦ research funds at the disposal of the laboratory leaders are allocated according to an internal algorithm based on scientific merit. These funds cover only current research expenses.

In 2001, there were 11 ongoing research projects financed by grants from the State Committee for Scientific Research (KBN), including three grants ordered by KBN.

Average financing of single research proposal in the frame of national granting system equals 10 000-20 000 EURO per year. On average, the grant holder teams consist of four persons.

The Institute teams are successful in securing support from competitive foreign granting systems. Table 2 summarises the support from international sources received by the Institute during the period 1999-2001.

No	Source of funding	Grant holder	Years	EURO
1	5 th Framework Programme	Dr. J. Dastych	2001-2003	231 703
2	5 th Framework Programme	Dr. L. Rychlewski	2001-2003	148 800
3	Johnson Institute and UCSD	Dr. L. Rychlewski	2000	49 250
4	UNESCO	Prof. M. Zylicz	2001-2002	32 833
5	UNESCO	Prof. J. Kuznicki	2001	10 944
6	PHARE SCI-TECH	Prof. J. Kuznicki	2000	4 000
7	Utrecht University	3 PhD fellowships	2000-2003	7 487
8	Max-Planck Institute (equipment)	Dr. M. Bochtler	2000-2001	664 621
9	Max-Planck Institute (JJRG)	Dr. M. Bochtler	2000-2005	613 496
10	Private donation (USA)	Prof. J. Kuznicki	1999	3 902
			Total	1 767 036

Table 2. Foreign grants

Staff at IIMCB

(as of March 2002)

Name	Function	Employer
1. Angelo Azzi	Director (till April 2001)	(IIMCB)
2. Jacek Kuznicki	Director (from February 2002)	(IIMCB, full)
3. Michal Witt	Deputy Director for scientific matters	(IIMCB, 1/2)
4. Vacant	Deputy Director for general matters	
5. Zbigniew Przygoda	Administration Manager	(IIMCB, 1/2)
6. Andrzej Sliwowski	Network Manager	(IIMCB, full)
7. Hanna Michalska	Financial Manager	(IIMCB, 1/2)
8. Iwona Marchewka	Accounting	(IIMCB, 1/2)
9. Renata Szymanczak	Accounting	(IIMCB, 1/2)
10. Agnieszka Ziemka	Scientific Secretary	(IIMCB, full)
11. Dorota Urbanowska	Scientific Secretary	(IIMCB, full)
12. Ewa Blazewicz	Secretarial assistant	(IIMCB, 3)

Department of Molecular Biology:

13. Maciej Zylicz	Head	(IIMCB+UNESCO fellowship)
14. Alicja Wawrzynow	Vice Head	(IIMCB+UNESCO fellowship)
15. Leszek Trzeciak	Postdoctoral Fellow	(IIMCB, full)
16. Frank King	PhD Student	(IBB+Kosciuszko Foundation, graduated in October, 2001)
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. Natalia Kunowska	PhD Student	(FNP)
22. Malgorzata Gutkowska	PhD Student	(FNP)
23. Dawid Walerych	MSc Student	(Volunteer)
24. Wanda Gocal	Technician	(IIMCB, full)
25. Grazyna Orleanska	Secretary	(IIMCB, full)

Laboratory of Molecular Immunology:

26. Jaroslaw Dastyh	Head	(IIMCB, full)
27. Urszula Bialek-Wyrzykowska	Postdoctoral Fellow	(Vth FP EU)
28. Dominika Trzaska	Research Assistant	(IIMCB, full)
29. Violetta Adamczewska	Research Assistant	(IIMCB, full)
30. Maciej Olszewski	Research Assistant	(Utrecht fellowship)

Laboratory of Bioinformatics:

31.Leszek Rychlewski	Head (till February 2002)	(IIMCB, 1/2)
32.Janusz M. Bujnicki	Head (from March 2002)	(IIMCB, 1/2)
33.Jakub Pas	PhD Student	(Volunteer)
34.Michal Kurowski	PhD Student	(Volunteer)
35.Zuzanna Stasinska	MSc Student	(Volunteer)
36.Marcin Feder	MSc Student	(Volunteer)

Laboratory of Molecular Neurology:

37.Michal Hetman	Head	(IIMCB, full)
38.Agata Gozdz	Research Assistant	(IIMCB, full)
39.Agata Habas	Research Assistant	(IIMCB, full)
40.Vacant	Postdoctoral Fellow	(IIMCB)

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

41.Matthias Bochtler	Head	(Max-Planck)
42.Roman Szczepanowski	Research Assistant	(IIMCB/Max-Planck)
43.Renata Filipek	Research Assistant	(IIMCB/Max-Planck)
44.Izabela Sabala	Research Assistant	(IIMCB/Max-Planck from March 2002)
45.Sergey Odintsov	PhD Student	(IIMCB/Max-Planck)

Laboratory of Neurodegeneration:

46.Jacek Kuznicki	Head	
47.Urszula Wojda	Postdoctoral Fellow	(IIMCB, from Jan 2002)
48.Cezary Zekanowski	Postdoctoral Fellow	(IIMCB, full)
49.Sanne Mikkelsen	Research Assistant	(PAN-Danish fellowship, till February 2002)
50.Tomasz Rudka	MSc Student	(Volunteer)
51.Malgorzata Mossakowska	Centenarians Project	(IIMCB, full)
52.Aleksandra Szybinska	Cell and DNA Bank	(IIMCB, full)

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

53.Katarzyna Starowicz	Research Assistant
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54.Barbara Ziolkowska	Research Assistant (from March 2002)
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