Academy of Sciences of the Czech Republic
Institute of Molecular Genetics

Scientific Report 2004 – 2005

Institute of Molecular Genetics
Academy of Sciences of the Czech Republic
Flemingovo nám. 2
166 37 Prague 6
Czech Republic

Telephone: (+420) 224 310 234
Fax: (+420) 224 310 955
E-mail: office@img.cas.cz
Home page: http//www.img.cas.cz

Director: Professor Václav Hořejší, Ph.D.

Contents

Scientific and Administrative Bodies of the Institute

Director: Professor Václav Hořejší, Ph.D.

1st Deputy Director and Scientific Secretary: Josef Geryk, Ph.D.

2nd Deputy Director: Professor Vladimír Viklický, M.D., Ph.D.

The Scientific Council of the Institute

Chairman: Petr Dráber, Ph.D., D.Sc.

Vice-Chairman: Jiří Hejnar, Ph.D.

Members: Ladislav Anděra, Ph.D., Petr Bartůněk, Ph.D., Professor Jan Bubeník, M.D., D.Sc., Professor Jiří Forejt, M.D., D.Sc., Professor Jitka Forstová, Ph.D., Professor Pavel Hozák, Ph.D., D.Sc., Zbyněk Kozmik, Ph.D., Professor Marie Lipoldová, Ph.D., Professor Zdena Palková, Ph.D., Peter Šebo, Ph.D.

Administrative and Technical Staff

Office of the Director

Head: Šárka Takáčová, M.A.

Secretaries: Leona Krausová, Gabriela Marešová

Economy Department

Head: Zdeňka Sokolová

Secretary: Táňa Rolníková

Finances: Milena Dobrá, Kateřina Drastilová, Jaroslava Samohylová, Karla Nosková, Miluše Šnajbergová, Emilie Štorchová

Technical and Material Supply: Věra Bálková, Ivana Brabencová, Hana Nezbedová

Personnel: Jitka Emanuelová

Director's Introduction

I became the new Director of the Institute of Molecular Genetics in a period of crucial importance – by the end of 2006, construction of our new, modern building in the Prague-Krč site of biomedical institutes of the Academy should be completed. Thus, at the beginning of 2007, almost all groups of our long-term divided Institute will move to work together in this new building. The Institute will then finally start to formally function as a standard, full-value scientific institution. I would like to express deep thanks to my predecessor, Professor Václav Pačes, who last year became President of the Academy of Sciences of the Czech Republic, for all his efforts that made it possible to start construction of our long desired new building.

I believe that namely after moving into the new building, we will possess all prerequisites to fulfil our goal – to become clearly the best biological research institution in the Czech Republic. A significant alteration of a part of scientific groups should contribute to this aim.

It is gratifying that our researchers continue to be successful in obtaining research grants, including prestigious international ones. At present, we have at our Institute three co-investigators of grants of the 6th Framework EC Programme, one NIH grant and one EMBO grant. The Institute hosts several „Research Centers" of the 1M and LC Programmes of the Ministry of Education, Youth and Sports of the Czech Republic. These Research Centers represent a crucial organizational and financial contribution for the Institute.

By January 1st, 2006, the Institute terminated publishing the journals Folia Biologica and Biology Letters, which, in my opinion, have lost their previous importance.

At present, 20 research groups of the Institute are dealing with several major topics including molecular and cellular biology, immunology, functional genomics and bioinformatics, study of oncogenes, molecular biology of development, molecular basis of fertilization, structural biology and mechanisms of receptor signalling. I consider as a very positive feature that the Institute hosts at present a total of 58 doctoral students and 29 undergraduates. Many of our scientists actively work as university teachers. All details of the Institute's activities are shown on our web pages (http://www.img.cas.cz/main.php?lang=en&subject=1).

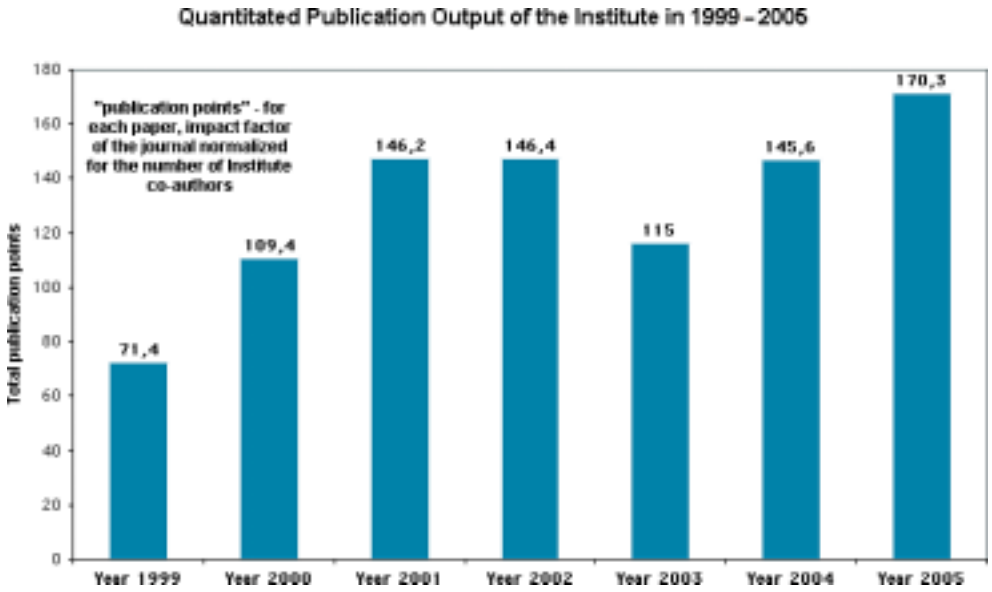
The high standing of the Institute researchers is testified by a number of awards and prizes, as shown on a following page. In 2004–2005 the Institute scientists were again authors or co-authors of publications in a number of prestigious international journals (e.g. Nature, Nat. Rev. Immunol., Nat. Rev. Genet., Nat. Genet., Immunity, J. Exp. Med., Proc. Natl. Acad. Sci. USA, Trends Immunol., Trends Genet., Mol. Cell Biol., Blood, Curr. Opin. Genet. Dev., J. Am. Chem. Soc., EMBO J.).

Although I consider basic research as our major priority, our Institute is also successful in valuable applied research leading to specific practical applications. These activities resulted in several well-prospering biotech spin-off companies.

The scientists of the Institute of Molecular Genetics are pleased to co-operate frequently with the media in popularizing scientific results and explaining the importance of science in general and molecular genetics in particular.

I am fully convinced that the Institute has a really good chance to continue the previous successful tradition represented by the names of Milan Hašek, Jan Svoboda, and a number of others.

Václav Hořejší



Prizes and Awards

Professor Jan Bubeník and his team:

Prize of the Minister of Health for Research and Development, December 2005

Professor Jiří Forejt:

Honorary J.G. Mendel Medal for Merits in Biological Sciences (awarded by the Academy of Sciences of the Czech Republic), April 2004
Member of the European Molecular Biology Organization (since 1999)
Member of the Learned Society of the Czech Republic (since 1999)

Professor Václav Hořejší:

Medal of Merit (awarded by the President of the Czech Republic), October 2004
Member of the Learned Society of the Czech Republic (since 2003)

Professor Václav Pačes:

Elected President of the Academy of Sciences of the Czech Republic, March 2005
Member of the European Molecular Biology Organization (since 1997)
Member of the Learned Society of the Czech Republic (founding member, 1994)

Professor Jan Svoboda:

Member of the European Molecular Biology Organization (since 1995)
Member of the Learned Society of the Czech Republic (founding member, 1994)

Dr. Zdeněk Trachtulec:

Otto Wichterle Award (awarded by the Academy of Sciences of the Czech Republic), May 2004

Dr. Jan Závada:

Honorary J.G. Mendel Medal for Merits in Biological Sciences (awarded by the Academy of Sciences of the Czech Republic), April 2004
Member of the European Molecular Biology Organization (since 1996)
Member of the Learned Society of the Czech Republic (founding member, 1994)

Team of authors (head: Václav Hořejší; members: Ladislav Anděra, Pavla Angelisová, Tomáš Brdička, Naděžda Brdičková, Jan Černý, Karel Drbal, Ivan Hilgert, Ondrej Horváth, Vladimír Kořínek, Jiří Špička):

Prize of the Academy of Sciences of the Czech Republic, May 2004

Visit of the President of the Czech Republic, Mr. Václav Klaus, at the Institute of Molecular Genetics on June 21, 2005

Prestigious Grants

Howard Hughes Medical Institute:

Regulatory Role of Membrane Microdomains in Mast Cell Signalling, awarded to Petr Dráber (2001–2005)

New Tools for Genome Scanning and Positional Cloning of Mouse Complex Traits Loci, awarded to Jiří Forejt (2001–2005)

Genetic Analysis of the Immune Response, awarded to Marie Lipoldová (2001– 2005)

Volkswagen Stiftung:

Nuclear Hormonal Receptors in Normal and Malignant Haemopoiesis, co-investigator Petr Bartůněk (2002–2005)

5th EU Framework Programme:

Rational Approaches towards Understanding and Overcoming HIV Protease Inhibitor Resistance, awaded to Juraj Sedláček (2003–2005)

6th EU Framework Programme:

Clinigene, European Clinical Gene Transfer Advisory Network, awarded to Jan Bubeník (2005–2009)

The Functional Role of Microtubule Phosphorylation in Plant Development and Environmental Stimuli Response, awarded to Pavel Dráber (2004–2007)

Human Monoclonal Antibodies from a Library of Hybridomas, awarded to Václav Hořejší (2005–2008)

EMBO/HHMI Scientist Award:

Haemopoietic Cell Differentiation, awarded to Petr Bartůněk (2004–2006)

NIH:

Consomic Strains between C57/BL/6 and PWD, awarded to Jiří Forejt (2004–2007)

Ministry of Education, Youth and Sports of the Czech Republic:

Center of Molecular and Cellular Immunology, awarded to Václav Hořejší (2000–2004, 2005–2009)

Center of Integrated Genomics, awarded to Václav Pačes (2000–2004)

Center of Applied Genomics, awarded to Václav Pačes (2005–2009)

Teaching

Semestral Courses:

Molecular Mechanisms of Apoptosis, Ladislav Anděra, Faculty of Science, Charles University, Prague

Three-Dimensional Structure Solution of Macromolecules, Jiří Brynda and Pavlína Řezáčová, Faculty of Science, Charles University, Prague

Anti-tumour Immunity, Jan Bubeník, Faculty of Science, Charles University, Prague

Immunology and Gene Therapy of Tumours, Jan Bubeník, 1st Faculty of Medicine, Charles University, Prague

Molecular Mechanisms of Morphogenesis, Lukáš Čermák, Faculty of Science, Charles University, Prague

Advances in Immunology, Jan Černý and Václav Hořejší, Faculty of Science, Charles University, Prague

Structure and Function of the Cytoskeleton, Pavel Dráber, Faculty of Science, Charles University, Prague

Molecular Genetics of the Mammalian Organism, Jiří Forejt, Faculty of Science, Charles University, Prague

Regulation Mechanisms of Immunity, Vladimír Holáň, Faculty of Science, Charles University, Prague

Immunology, Václav Hořejší, Faculty of Science, Charles University, Prague

Molecular Immunology, Václav Hořejší, Faculty of Science, Charles University, Prague

Gene Expression, Jiří Jonák, 1st Faculty of Medicine, Charles University, Prague

Seminars „Biological Oxidation“, Jiří Jonák, 1st Faculty of Medicine, Charles University, Prague

Biochemistry of Animal Reproduction, Věra Jonáková, Faculty of Science, Charles University, Prague

Mechanisms of Cell Proliferation, Jan Kovář, Faculty of Science, Charles University, Prague

Model Organisms in Developmental Biology, Zbyněk Kozmik, Faculty of Science, Charles University, Prague

Advances in Immunology of Infectious Diseases. Molecular Mechanisms of Defence against Infection, Marie Lipoldová, 3rd Faculty of Medicine, Charles University, Prague

Bioinformatics, Jan Pačes (with Jiří Vondrášek from the Institute of Organic Chemistry and Biochemistry AS CR), Faculty of Science, Charles University, Prague

Bioinformatics, Jan Pačes, University of Perugia, Perugia, Italy

Molecular Genetics, Václav Pačes, Institute of Chemical Technology, Prague

Gene Engineering, Václav Pačes (with Tomáš Ruml from the Institute of Chemical Technology), Institute of Chemical Technology, Prague

Molecular Mechanisms of Fertilization, Jana Pěknicová, Faculty of Science, Charles University, Prague

Molecular and Cellular Oncology, Jan Svoboda and Jan Závada, Faculty of Science, Charles University, Prague

Biotechnology of Monoclonal Antibodies, Vladimír Viklický, Faculty of Science, Charles University, Prague

System of Funding Research and Development in the Czech Republic, Vladimír Viklický, Faculty of Science, Charles University, Prague

Diploma Theses:

Alexandra Bukovská Effect of the Src Protein on p70 S6 Kinase Activity (*Supervisor:* Zdena Tuháčková, 2005, Faculty of Science, Charles University, Prague)

Viktor Bugajev Changes in Composition and Expression of Membrane Components in Mast Cell Activation Measured by Flow Cytometry (*Supervisor:* Petr Dráber, 2005, Faculty of Science, Charles University, Prague)

Eva Cibulková Study of the Properties of Boar Seminal Plasma Hyaluronidase (*Supervisor:* Věra Jonáková, 2005, Faculty of Science, Charles University, Prague)

Ľubomíra Fričová Transcriptional Suppression of Integrated Retroviruses by Overexpression of DNA Methyltransferases Dnmt1 and Dnmt3a (*Supervisor:* Jiří Hejnar, 2005, Faculty of Science, Charles University, Prague)

David Homolka Expression Analysis of Selected X-Linked Genes during Mouse Spermatogenesis
(*Supervisor:* Petr Jansa, 2004, Faculty of Science, Charles University, Prague)

Dagmar Hýblová Antibodies against G-Protein-Linked Receptors
(*Supervisor:* Blanka Železná, 2004, Faculty of Science, Charles University, Prague)

Jan Kosla Transport of v-Myb Proteins to Cells via TAT Transduction Domain
(*Supervisor:* Michal Dvořák, 2005, Faculty of Science, Charles University, Prague)

Roman Lištinský Role of the PAG Adaptor Protein in Cell Proliferation
(*Supervisor:* Václav Hořejší, 2005, Faculty of Science, Charles University, Prague)

Magda Matoušková Analysis of 5' LTR Methylation of Syncytin – the Human Gene of Retroviral Origin (*Supervisor:* Jiří Hejnar, 2004, Faculty of Science, Charles University, Prague)

Marie Pexidrová Localization of - and -Tubulin in Patients with Normal and Pathological Spermiograms
(*Supervisor:* Jana Pěknicová, 2004, Faculty of Science, Charles University, Prague)

Luděk Sojka The *ybx*F Gene – a New Component of the Streptomycin Receptor in *Bacillus subtilis*
(*Supervisor:* Jiří Jonák, 2004, Faculty of Science, Charles University, Prague)

Jan Švadlenka Identification and Characterization of Proteins Interacting with the N-terminal Part of the Daxx Protein
(*Supervisor:* Ladislav Anděra, 2005, Faculty of Science, Charles University, Prague)

Ph.D. Theses:

Jana Krešlová Transcription Factors and Their Role in Animal Development and Evolution
(*Supervisor:* Zbyněk Kozmik, 2004, Faculty of Science, Charles University, Prague)

Vitaliy Kukharskyy Role of Tyrosin Kinases Src and Syk Families in Intracellular Signal Transduction with Participation of Microtubules
(*Supervisor:* Pavel Dráber, 2004, Faculty of Science, Charles University, Prague)

Vendula Kyselová Effect of Endocrine Disruptors and Genetic Factors on Mouse Fertility
(*Supervisor:* Jana Pěknicová, 2004, Faculty of Science, Charles University, Prague)

Lenka Libusová Immunolocalization and Function of Microtubular Proteins in Selected Protozoan Species
(*Supervisor:* Pavel Dráber, 2005, Faculty of Science, Charles University, Prague)

Romana Mikyšková Therapy of HPV16 Virus E6/E7 Oncogene-Induced Mouse Tumours using Vaccines with Inserted Cytokine Genes and Dendritic Cell-Based Vaccines
(*Supervisor:* Jan Bubeník, 2004, 3rd Faculty of Medicine, Charles University, Prague)

Iva Rohoušová Sandfly Saliva and Host Immune Response
(*Supervisor:* Marie Lipoldová, 2005, Faculty of Science, Charles University, Prague)

Pavlína Řezáčová Structural Basis of HIV Proteases Inhibition by a Monoclonal Antibody
(*Supervisor:* Jiří Brynda, 2004, Faculty of Science, Charles University, Prague)

Petra Slavíčková Study of the Characteristics and Functions of Boar and Bull Seminal Plasma Proteins
(*Supervisor:* Věra Jonáková, 2004, Faculty of Science, Charles University, Prague)

Helena Tolarová Actin in Mast Cell Signalling
(*Supervisor:* Petr Dráber, 2005, Faculty of Science, Charles University, Prague)

Jaroslav Truksa Molecular Mechanisms of Apoptosis Induction in Cancer Cells
(*Supervisor:* Jan Kovář, 2005, Faculty of Science, Charles University, Prague)

Tomáš Vacík Ts43H Segmental Trisomy, a New Mouse Model of Aneuploidy Syndromes
(*Supervisor:* Jiří Forejt, 2004, Institute of Chemical Technology, Prague)

Lectures and Seminars by Visiting Scientists

Thirty-four seminars were given at the Institute of Molecular Genetics by visiting scientists from other research organizations around the world within the years 2004 and 2005. Speakers came from ten foreign countries – Austria, Brazil, Bulgaria, France, Germany, Israel, Italy, Sweden, the United Kingdom, and the United States. In addition, there were numerous informal seminars at which the Institute's members spoke about their own research.

Listed below are the seminars presented by the visiting scientists:

Dr. Hiroshi Arakawa (Institute of Radiology, Munich): Target Site Deletion in Chicken DT40 Cells as a New Tool for Studies on Generation of Diversity in Immune Cells. January 17, 2004.

Jacky Cosson, Ph.D. (CNRS, URA 671, Villefrance sur Mer): Motility of Fish Spermatozoa. March 23, 2004.

Dr. Radek Blatný (Institute of Immunology, Medical University Vienna and BMT Bio Molecular Therapeutics, GmbH, Vienna): Construction of Truncated Forms of CD31 Receptor Globulin. April 7, 2004.

Dr. Elana Miriami (The Hebrew University of Jerusalem): Detecting Intronic cis-Regulatory Elements for Alternative Splicing by Computational Analysis. April 9, 2004.

Martin Balaštík (University of Göttingen): Keeping Neurons in Good Trim: TRIM-RING Finger Proteins in Neurodegeneration. May 11, 2004.

Professor Keith Latham (Department of Biochemistry Temple University School of Medicine, Philadelphia, PA): Somatic Cell-Like Features of Cloned Embryos. May 13, 2004.

Professor E. Peter Geiduschek (UC San Diego, CA): Archaeal Transcription from a Bacterial and Eukaryotic Perspective. June 2, 2004.

Dr. Serge Roche (CRBM, CNRS FRE2593, Montpellier): Src Tyrosine Kinases in Signal Transduction and Cell Growth Control. June 28, 2004.

Professor Rayna Georgieva (Institute of Biology and Immunology of Reproduction, Sofia): Role of Selected Sperm Proteins in Reproduction. June 25, 2004.

Professor Robert Williams (Department of Anatomy and Neurobiology, Pediatrics Center for Genomics and Bioinformatics, University of Tennessee Health Science Center, Memphis, TN): The Complexity of Molecular Networks Modulating Gene Expression in the Rodent Brain: Use of RI strains. August 20, 2004.

Dr. Weiguo Zhang (Duke University, Durham, NC): Transmembrane Adaptor Proteins in Leukocyte Signaling. September 10, 2004.

Dr. Andrew Pearce, Ph.D. (Centre for Cardiovascular Studies, Institute of Biomedical Research, Birmingham): Comparison of the Activation Ability of Platelets from Normal Mice and Mice Defective in Adaptor Proteins LAT, NTAL, or Both These Proteins. September 26 – October 1, 2004.

Professor Mark J. Federspiel, Ph.D. (Mayo Clinic College of Medicine, Rochester, MN): Avian Leukosis Virus Envelope Glycoproteins: A model for Studying Retrovirus Entry and a Eukaryotic Platform for Polypeptide Display Technology. October 11, 2004.

Dr. Daniel Zicha (Department of. Light Microscopy, Cancer Research Institute, London): Novel Approaches of Light Microscopy to the Study of Genetically Defined Complex Functions. December 20, 2004.

Dr. Heike Bantel (Department for Gastroenterology, Hepatology and Endocrinology, Medical School, Hannover): Caspase Activity in Serum and Liver Explants. September 22, 2005.

Dr. Vladimír Beneš (European Molecular Biology Laboratory, Heidelberg): Functional Genomics at EMBL – Technology Point of View. May 23, 2005.

Dr. Josef Bodor (Dana-Farber Cancer Institute, Harvard University, Boston, MA): Transcriptional Attenuation of IL-2 and Its Role in Regulatory T Cells. May 9, 2005.

Dr. Christian Boulin (European Molecular Biology Laboratory, Heidelberg): EMBL and Development of Its Scientific Core Facilities. May 23, 2005.

Dr. Tomáš Brdička (University of California, San Francisco, CA): Negative Regulatory Switch in ZAP-70: Analogy with Receptor Tyrosine Kinases. February 28, 2005.

Dr. Jacky Cosson (Université Pierre at Marie Curie, Observatoire Océanologique, Villefrance sur Mer): Flagella in Silico – Building and Driving Computer Axonemal Functions. March 21, 2005.

Dr. Zbigniew Dauter (Synchrotron Radiation Research Section, National Cancer Institute, Argonne National Laboratory, Argonne, IL): The Principles of Biological Crystallography. How to Read and Interpret the International Tables. October 10, 2005.

Dr. Cornelius Gross (European Molecular Biology Laboratory, Monterotondo): Genetic Screen for Susceptibility Loci for Common Diseases using Chromosomal Dosage Analysis. July 13, 2005.

Professor Dr. Eva Klein (Microbiology and Tumor Biology Center (MTC), Karolinska Institutet, Stockholm): Epstein-Barr Virus (EBV) – B Cell Relationships. October 31, 2005.

Professor Dr. George Klein (Microbiology and Tumor Biology Center (MTC), Karolinska Institutet, Stockholm): Non-immune Surveillance against Tumors. December 31, 2005.

Professor David A. Lawrence (Wadsworth Center, NYS Dept. of Health, NY): Immune Responses Modulate Neuronal Functions. May 18, 2005.

Dr. Jens Mayer (University of Saarland, Homburg): Status, Evolution and Transcription of Human Repetitive DNA Sequences. October 18, 2005.

Professor Massimo Palmarini (Faculty of Veterinary Medicine, University of Glasgow): Blocking the Exit: a New Twist in the Never-ending Battle between Endogenous and Exogenous Retroviruses. May 3, 2005.

Professor Paula M. Pitha-Rowe (Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD): Cell Defense against Virus Offense. May 25, 2005.

Dr. Paolo Salomoni (MRC Toxicology Unit, Leicester): The Role of Daxx in Stress-Induced Cell Death. February 7, 2005.

Dr. Peter Sicinski (Dana-Farber Cancer Institute, Boston, MA): Cell Cycle Machinery in Mouse Development and in Cancer. April 2, 2005.

Dr. Antonio R. L. Teixeira (Faculty of Medicine, University of Brasilia): Chagas: a Parasite Vector Genetic Disease. March 30, 2005.

Prof. Eberhard Unger (Institute of Molecular Biotechnology, Jena): Microtubules, Kinesins and Nanoparticle Decoration. July 1, 2005.

Prof. Jürgen Wienands (University Tübingen): B-cell Signaling in Health and Disease. December 16, 2005.

Dr. Daniel Zicha (Cancer Research UK London Research Institute, London): Microarrays and High Content Screening in Studies of Metastasis using a Sarcoma Model in Inbred LEWIS Rats. April 30, 2005.

Research Groups of the Institute

Molecular Immunology
Head: Václav Hořejší
horejsi@biomed.cas.cz

Molecular and Cellular Immunology
Head: Marie Lipoldová
lipoldova@img.cas.cz

Tumour Immunology
Head: Milan Reiniš
reinis@img.cas.cz

Transplantation Immunology
Head: Alena Zajícová
zajicova@img.cas.cz

Genomics and Bioinformatics
Head: Čestmír Vlček
vlcek@img.cas.cz

Mammalian Molecular Genetics
Head: Jiří Forejt
jforejt@biomed.cas.cz

Cellular and Viral Genetics
Head: Jiří Hejnar
hejnar@img.cas.cz

Molecular Virology
Head: Michal Dvořák
mdvorak@img.cas.cz

Transcriptional Regulation
Head: Zbyněk Kozmik
kozmik@img.cas.cz

Cell Differentiation
Head: Petr Bartůněk
bartunek@img.cas.cz

Cell Signalling and Apoptosis
Head: Ladislav Anděra
andera@biomed.cas.cz

Signal Transduction
Head: Petr Dráber
draberpe@biomed.cas.cz

Intracellular Communications
Head: Zdena Tuháčková
tuhack@img.cas.cz

Biology of Cytoskeleton
Head: Pavel Dráber
paveldra@biomed.cas.cz

Cell Biology
Head: Eva Matoušková
matous@img.cas.cz

Gene Expression
Head: Jiří Jonák
jjon@img.cas.cz

Micromorphology of Biopolymers
Head: Jan Korb
korb@img.cas.cz

Recombinant Expression and Structural Biology
Head: Juraj Sedláček
sedlacek@img.cas.cz

Biology and Biochemistry of Fertilization
Head: Jana Pěknicová
jpeknic@biomed.cas.cz

Biochemistry of Reproduction
Head: Věra Jonáková
vjon@img.cas.cz

Molecular Immunology

Head: Václav Hořejší

Research Fellows: Pavla Angelisová, Karel Drbal

Graduate Student: Jiří Špička

Research Assistant: Ondrej Horváth

Undergraduate Students: Tereza Havlová, Linda Turková, Peter Dráber (from September 2005), Lukáš Chmátal (from April 2005), Roman Lištinský (until May 2005)

Technicians: Jana Doškářová (until April 2005), Jana Pokorná, Kateřina Zachová (from April 2005)

Secretary: Eva Tvrzníková (part time)

Introduction

A major topic of our laboratory has been for many years structure and function of human leukocyte surface molecules. Many membrane (glyco)proteins and glycolipids are of essential importance for all aspects of leukocyte biology and functioning of the immune system. A number of them remain probably so far undiscovered and functions and mutual interactions among the known ones are only partly understood. In recent years we have been dealing mainly with membrane microdomains called lipid rafts, termed also GEMs (glycolipid-enriched membrane microdomains). These membrane structures are distinguished from the rest of the membrane by their high content of glycosphingolipids, cholesterol and glycolipid (GPI)-anchored proteins, while transmembrane proteins are mostly excluded from them. Importantly, they accumulate cytoplasmic signalling molecules such as heterotrimeric G-proteins and Src-family protein tyrosine kinases. These membrane „signalling islets“ appear to be of crucial importance for immunoreceptor signalling. Because of the functional importance of lipid rafts (which were actually co-discovered by our laboratory fifteen years ago) we are interested in thorough functional characterization of their protein components, namely transmembrane adaptor proteins (TRAPs) discovered previously by us (PAG/Cbp, NTAL/LAB, LIME), and their involvement in immunoreceptor signalling. Our work has been based on collaborations with several laboratories abroad.

Results

In 2004–2005 we participated in construction and characterization of mice deficient in the transmembrane adaptor protein NTAL (gene knock-out); based on these studies, NTAL appers to be mainly a negative regulator of immunoreceptor signalling (3,5,15). Also, we participated in studies demonstrating that TRAPs may be useful immunohistochemical markers of specific lymphoid tissues and some lymphomas (14,20). Furthermore, we collaborated on development of monoclonal antibodies to “empty” MHC class II molecules that may play important roles in an alternative way of antigen presentation (1,10).

As can be seen from the publications list below, we also wrote several reviews (6–8) and participated in several collaborative studies concerning membrane rafts and their components, MHC proteins and other leukocyte membrane proteins.

During the last 20 years, our laboratory developed a large series of monoclonal antibodies to a number of leukocyte molecules and signalling proteins (e.g. MHC class I and class II, HLA-G, HLA-E, CD2, 3, 4, 5, 6, 7, 8, 9, 10, 11a, 11b, 14, 15, 16, 17, 18, 20, 22, 25, 29, 31, 43, 44, 45, 45RA, 45RB, 46, 47, 48, 50, 53, 54, 55, 56, 58, 59, 63, 71, 80, 97, 98, 99, 105, 108, 147, 173, 177 and 222; P-Tyr, Lck, Fyn, Csk, TRIM, SIT, LAT, PAG, NTAL, LIME, EBP50, SLP-76, ZAP-70). These monoclonals are available to other researchers either directly on request or commercially through a spin-off company of our Institute, Exbio Ltd. (www.exbio.cz).

The work of the group was supported by the Academy of Sciences of the Czech Republic (institutional project No. AV0Z50520514), the Ministry of Education, Youth and Sports of the Czech Republic (consecutive projects Center of Molecular and Cellular Immunology (LN00A0026 and 1M68378050001)), international collaboration projects EUREKA No. 2334 COCANAL and 1P04OE190 RECAN.

Publications

1. Carven, G.J, Chitta, S., Hilgert, I., Rushe, M. M., Baggio, R.F., Palmer, M., Arenas, J.E., Strominger, J.L., Hořejší, V., Santambrogio, L., Stern, L.J.: Monoclonal antibodies specific for the empty conformation of HLA-DR1 reveal the conformational change associated with peptide binding. *J. Biol. Chem. 279: 16561-16570, 2004.*

2. Tkaczyk, C., Hořejší, V., Iwaki, S., Dráber, P., Samelson, L.E., Satterthwaite, A.B., Nahm, D.H., Metcalfe, D.D., Gilfillan, A.M.: NTAL phosphorylation is a pivotal link between the signaling cascades leading to human mast cell degranulation following Kit activation and Fc RI aggregation. *Blood 104: 207-214, 2004.*

3. Volná, P., Lebduška, P., Dráberova, L., Šímová, Š., Heneberg, P., Boubelík, M., Bugajev, V., Malissen, B., Wilson, B.S., Hořejší, V., Malissen, M., Dráber, P.: Negative regulation of mast cell signaling and function by the adaptor LAB/NTAL. *J. Exp. Med. 200: 1001-1013, 2004.*

4. Bařinka, C., Mlčochová, P., Sacha, P., Hilgert, I., Majer, P., Slusher, B.S., Hořejší, V., Konvalinka, J.: Amino acids at the N- and C-termini of human glutamate carboxypeptidase II are required for enzymatic activity and proper folding. *Eur. J. Biochem. 271: 2782-2790, 2004.*

5. Stork, B., Engelke, M., Frey, J., Hořejší, V., Hamm-Baarke, A., Schraven, B., Kurosaki, T., Wienands, J.: Grb2 and the Non-T Cell Activation Linker NTAL constitute a Ca(2+)-regulating signal circuit in B lymphocytes. *Immunity 21: 681-691, 2004.*

6. Hořejší, V.: Transmembrane adaptor proteins in membrane microdomains: important regulators of immunoreceptor signaling. *Immunol. Lett. 92: 43-49, 2004.*

7. Hořejší, V., Zhang, W., Schraven, B.: Transmembrane adaptor proteins: organizers of immunoreceptor signalling. *Nat. Rev. Immunol. 4: 603-616, 2004.*

8. Hořejší, V.: Lipid rafts and their roles in T cell activation. *Microbes and Infection. 7: 310-316, 2005.*

9. Brdička, T., Kadleček, T.A., Roose, J.P., Pastuszak, A.W., Weiss, A.: Intramolecular regulatory switch in ZAP-70: analogy with receptor tyrosine kinases. *Mol. Cell Biol. 25: 4924-4933, 2005.*

10. Potolicchio, I., Chitta, S., Xu, X., Fonseca, D., Crisi, G., Hořejší, V., Strominger, J.L., Stern, L.J., Raposo, G., Santambrogio, L.: Conformational variation of surface class II MHC proteins during myeloid dendritic cell differentiation accompanies structural changes in lysosomal MIIC. *J. Immunol. 175: 4935-4947, 2005.*

11. Gonen-Gross, T., Achdout, H., Arnon, T.I., Gazit, R., Stern, N., Hořejší, V., Goldman-Wohl, D., Yagel, S., Mandelboim, O.: The CD85J/leukocyte inhibitory receptor-1 distinguishes between conformed and beta2-microglobulin-free HLA-G molecules. *J. Immunol. 175: 4866-4874, 2005.*

12. Fronková, E., Krejčí, O., Kalina, T., Horváth, O., Trka, J., Hrušák, O.: Lymphoid differentiation pathways can be traced by TCR delta rearrangements. *J. Immunol. 175: 2495-2500, 2005.*

13. Leksa, V., Godar, S., Schiller, H.B., Fuertbauer, E., Muhammad, A., Slezaková, K., Hořejší, V., Steinlein, P., Weidle, U.H., Binder, B.R., Stockinger, H.: TGF- -induced apoptosis in endothelial cells mediated by M6P/IGFII-R and mini-plasminogen. *J. Cell Sci. 118: 4577-4586, 2005.*

14. Švec, A., Velenská, Z., Hořejší, V.: Expression pattern of adaptor protein PAG: Correlation between secondary lymphatic follicle and histogenetically related malignant lymphomas. *Immunol. Lett. 100: 94-97, 2005.*

15. Wang, Y., Horváth, O., Hamm-Baarke, A., Richelme, M., Gregoire, C., Guinamard, R., Hořejší, V., Angelisová, P., Špička, J., Schraven, B., Malissen, B., Malissen, M.: Single and combined deletions of the NTAL/LAB and LAT adaptors minimally affect B-cell development and function. *Mol. Cell Biol. 25: 4455-4465, 2005.*

16. Gesierich, S., Paret, C., Hildebrand, D., Weitz, J., Zgraggen, K., Schmitz-Winnenthal, F.H., Hořejší, V., Yoshie, O., Herlyn, D., Ashman, L.K., Zoller, M.: Colocalization of the tetraspanins, CO-029 and CD151, with integrins in human pancreatic adenocarcinoma: impact on cell motility. *Clin. Cancer Res. 11: 2840-2852, 2005.*

17. Swart, B, Salganik, M.P., Wand, M.P., Tinckam, K., Milford, E.L., Drbal, K., Angelisová, P., Hořejší, V., Macardle, P., Bailey, S., Hollemweguer, E., Hodge, G., Nairn, J., Miliard, D., Dagdeviren, A., Dandie, G.W., Zola, H.; HLDA8 blind panel.: The HLDA8 blind panel: findings and conclusions. *J. Immunol. Methods 305: 75-83, 2005.*

18. Zola, H., Swart, B., Nicholson, I., Aasted, B., Bensussan, A., Boumsell, L., Buckley, C., Clark, G., Drbal, K., Engel, P., Hart, D., Hořejší, V., Isacke, C., Macardle, P., Malavasi, F., Mason, D., Olive, D., Saalmueller, A., Schlossman, S.F., Schwartz-Albiez, R., Simmons, P., Tedder, T.F., Uguccioni, M., Warren, H.: CD molecules 2005: human cell differentiation molecules. *Blood 106: 3123-3126, 2005.*

19. Horváth, O., Drbal, K., Angelisová, P., Hilgert, I., Hořejší, V.: Non-lineage antigens: Section report. *Cell Immunol. 236: 42-47, 2005.*

20. Tedoldi, S., Paterson, J.C., Hansmann, M.L., Natkunam, Y., Ruediger, T., Angelisová, P., Du, M.Q., Roberton, H., Roncador, G., Sanchez, L., Pozzobon, M., Masir, N., Barry, R., Pileri, S., Mason, D.Y., Marafioti, T., Hořejší, V.: Transmembrane adaptor molecules: A new category of lymphoid cell markers. *Blood 107: 213-221, 2006*.

Molecular and Cellular Immunology

Head: Marie Lipoldová

Research Fellows: Helena Havelková, Jarmila Vojtíšková

Graduate Students: Igor Grekov[1], Elena Gusareva[1], Tetyana Kobets[2], Iryna Kurey[1], Tomáš Pilčík[2], Iva Rohoušová[3], Martina Slapničková[1] (maternity leave until September 2005)

Senior Research Assistant: Marie Kejmarová

Technicians: Monika Buddeusová, Ivan Novozámský

Undergraduate Students: Michaela Cardová, Lenka Kronová, Marie Vanišová

Former Colleagues: Jana Badalová, Vladimir Vladimirov, Igor Kárník

[1] Supported in part by 3rd Faculty of Medicine, Charles University, Prague
[2] Supported in part by the Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague
[3] Ph.D. student of the Department of Parasitology, Faculty of Science, Charles University, Prague

Introduction

The research programme of the laboratory follows three main topics:

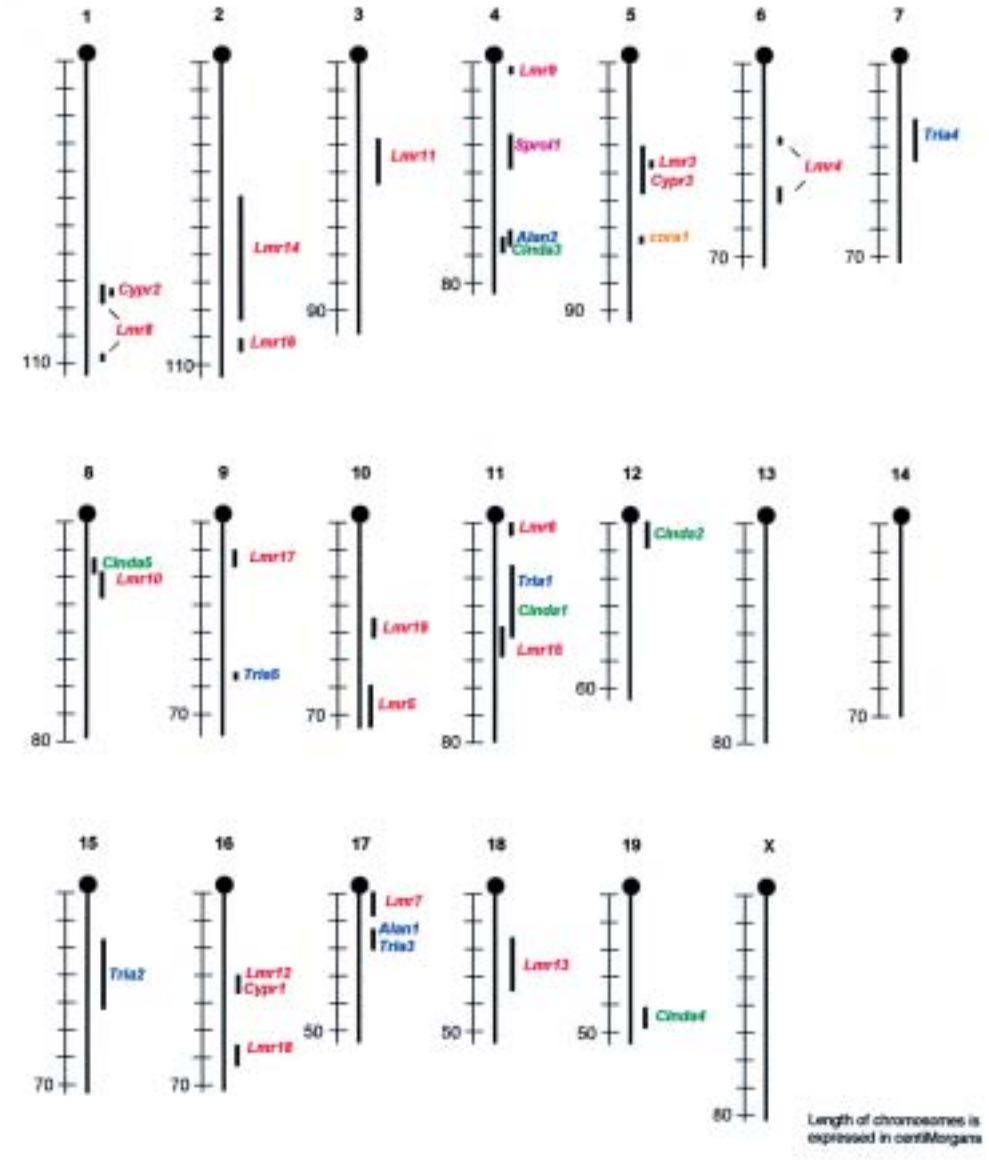
- **mapping and functional analysis of genes that control resistance to infection**
- **activation of lymphocytes and cancer susceptibility**
- **genetic and environmental influence on atopy**

We apply a special tool for genetic analysis of multigenically-controlled biological traits: the Recombinant Congenic Strains (RCS). The series of RCS comprises 20 homozygous mouse strains. Each RC strain contains a different, random, set of about 12.5% genes from a parental donor strain and about 87.5% genes of a parental background strain. In this way, the individual genes of the donor strain participating in the complex control of multigenically-controlled biological traits become divided into different RC strains, where they can be studied one by one. Thus, the RCS system transforms a multigenic difference into a set of single gene differences (or oligogenic differences). Using this system, we have separated and mapped 34 novel loci that control immune response and resistance to infection (Fig. 1).

Results

Mapping and functional analysis of genes that control resistance to infection

Leishmaniasis in humans is a major health problem in a number of countries and the basis of heterogeneity of its clinical manifestations and underlying immunological responses is not understood. The disease is transmitted to vertebrates by phlebotomine sandflies and is caused by protozoan parasites of genus *Leishmania* that infects mononuclear phagocytes of the vertebrate host. Infection of some strains of mice with *Leishmania major* closely mimics human infection due to *Leishmania donovani* (kala-azar). In most mouse strains *L. major* infection causes no



Chromosome location of mouse loci controlling immune response which were mapped by our laboratory

Alan (Alloantigen response)
Alan 1 (Immunogenetics, 51: 755, 2000)
Alan 2 (Genes and Immunity, 1: 483, 2000)

Cinda (Cytokine-induced activation)
Cinda 1, 2 (Genomics 42:11, 1997)
Cinda 3, 4, 5 (Int J Cancer 114:394-399, 2005)

cora (correlation)
cora 1 (Immunogenetics 49: 134, 1999)

Cypr (Cytokine production)
Cypr 1-3 (Immunogenetics 49: 134, 1999)

Lmr (Leishmania major response)
Lmr 3-7 (Genes and Immunity, 1: 200, 2000)
Lmr 3, 5, 8-14 (Genes and Immunity, 3: 187, 2002)
Lmr 13-15 (Infection and Immunity 71: 2041, 2003)
Lmr 3, 5, 8-19 (Genes and Immunity 7: in press 2006)

Sprol (Spontaneous proliferation)
Sprol 1 (Mammalian Genome 10:670, 1999)

Tria (T cell receptor-induced activation)
Tria 1, 2 (Immunogenetics 44: 475, 1996)
Tria 3 (Immunogenetics 49: 435, 1999)

or only transient pathological changes, whereas some strains develop a systemic visceral disease with spleno- and hepatomegaly, anaemia, hypergammaglobulinaemia and skin lesions. As the outcome of infection by *L. major* in the mouse is considerably dependent on the inherited characteristics of the host, the mouse models are suitable for study of clinical and immunological heterogeneity of leishmaniasis. However, although considerable data on the immune response has been obtained, the genetic basis of the disease heterogeneity and its relationship to immunological reactivity remain unclear.

i) Characterization of *Lmr* genes can contribute to our understanding of susceptibility to infectious diseases in general

We have shown that the susceptibility to *L. major* is multigenically controlled (Science 274: 1392, 1996) and detected and mapped seventeen novel *Lmr* (*Leishmania major* response) genes that control the symptoms of infection in the strains CcS-5, -16 and -20. We have also calculated the proportion of total variance explained by individual *Lmr* loci (5). In each strain, one or two loci with strong effects were detected, whereas the other loci identified have weaker influences on pathology. Surprisingly, the individual *Lmr* loci control thirteen different combinations of pathological and immunological symptoms. Seven loci control both pathological and immunological parameters, ten influence immunological parameters only. Importanly, these studies revealed previously unknown organ-specific genetic control of anti-*Leishmania* responses. Elucidation of the basis of this phenomenon can potentially lead to improvement of therapy of patients with different symptoms.

Our data provide perhaps the most extensive documentation of heterogeneity of gene effects in infectious disease, although a small number of examples has been described before. For example, it was observed in *L. donovani* infection, where the genes *Slc11a1* (Solute Carrier Family 11, member 1) and *Lyst* (lysosomal trafficking regulator) have different influences on organ pathology. Similarly, organ-specific genetic effects have been indicated in studies of *Trypanosoma cruzi* infection. Heterogeneity has been extensively studied in the response to *Borrelia burgdorferi*, in which different genes control distinct clinical symptoms and immunoglobulin subtypes, and in the response to *Toxoplasma gondii*, in which different host loci control parasite burden in the brain and survival of the host (reviewed in 6). Some *Lmr* loci co-localize with QTLs that influence the response to other infectious agents, including both bacteria and parasites (5, 6). This suggests the presence either of clusters of functionally related genes, or of genes that are involved in controlling the response to several infections.

ii) Influence of sandfly saliva on immune response

We have also analysed influence of sandfly saliva of phlebotomine vectors of *Leishmania* parasites on immune functions (2). Saliva of sandflies (Diptera: Phlebotominae) plays an important role in transmission of *Leishmania* parasites by modulating the host immune response. We have therefore analysed and compared the immunomodulation effects of salivary gland lysate (SGL) of three different sandflies: *Phlebotomus papatasi*, *P. sergenti* or *Lutzomyia longipalpis*. Concanavalin A-stimulated proliferation of splenocytes and IFN- production was significantly suppressed with SGLs of all three sandfly species. SGL to a lesser degree also impaired production of IL-2 and IL-4. Despite some species-specific differences in the intensity of immunomodulatory effects, saliva of all sandfly species modulated cell proliferation as well as cytokine production in a similar way.

Activation of lymphocytes and cancer susceptibility

Novel genotyping and statistical tools have led to mapping of numerous QTL (quantitative trait lo-

ci) for multigenic traits that previously could not be detected. The relationships of these QTL families to other QTL families and the functional specialization of their members can now be studied. We have mapped a number of loci controlling activation of T lymphocytes by mitogens and cytokines and their capacity to produce cytokines. In the strain OcB-9, we mapped three novel loci controlling proliferative T-cell response to cytokines IL-2 and IL-4 (*Cinda3*) or IL-4 only (*Cinda4* and *Cinda5*). These novel *Cinda* loci and the previously mapped *Cinda1* locus seem to be located in genomic regions together with other QTL families: macrophage function loci *Marif1* and *Marif2*, proteoglycan-induced arthritis loci *Pgia4*, *Pgia7* and *Pgia12* and lung tumour susceptibility loci *Sluc1*, *Sluc4*, *Sluc6* and *Sluc20*. The possible relevance of these QTL associations in several different sites of the genome for the immune response, inflammation and tumorigenesis has to be elucidated (1).

Environmental influence on atopy

Major allergens and prevalence in sensitization in atopic patients vary in different populations, providing cues about the pathogenic effects of the environment and lifestyle. We estimated the specificity and intensity of sensitization to 20 different airborne allergens in children and adolescent patients with atopic bronchial asthma from Tomsk and Thumen, cities of west Siberia, Russia. Cat (e1) was found to be the major allergen with 57.3% of Russian asthmatic patients sensitized to this allergen. Other important allergens were mites *Dermatophagoides pteronyssinus* (d1), *D. farinae* (d2) and dog allergen (e2), with more than 30% of asthmatics sensitized.
This is the first report about the distribution of specific IgE to airborne allergens in the asthmatic patients from the Russia (4).

Collaborations:

The RCS system and the support with genetic mapping is provided by Prof. Peter Demant (Roswell Park Cancer Institute, Buffalo, NY, U.S.A.). In *L. major* infection studies we collaborate with Assoc. Prof. M. Svobodová and Prof. P. Volf (Department of Parasitology of the Faculty of Science, Charles University, Prague). In the studies of allergy we collaborate with Prof. L.M. Ogorodova (Siberian State Medical University, Tomsk, Russia) and Prof. V.P. Puzyrev (Scientific Research Institute of Medical Genetics of the Tomsk Scientific Centre of the Siberian Branch of the Russian Academy of Medical Sciences).

In the years 2004 and 2005, the work of the laboratory was supported by grants Nos. 310/03/1381 and 310/03/H147 from the Grant Agency of the Czech Republic and by the Howard Hughes Medical Institute (HHMI 55000323).

Publications

1. Lipoldová, M., Havelková, H., Badalová, J., Demant, P.: Novel loci controlling lymphocyte proliferative response to cytokines and their clustering with loci controlling autoimmune reactions, macrophage function, and lung tumor susceptibility. *Int. J. Cancer 114: 394-399, 2005.*

2. Rohoušová, I., Volf, P., Lipoldová, M.: Modulation of murine cellular immune response and cytokine production by salivary gland lysate of three sandfly species. *Parasite Immunol. 27: 469-473, 2005.*

3. Havelková, H., Holáň, V., Kárník, I., Lipoldová, M.: Mouse model for analysis of non-MHC genes that influence allogeneic response: recombinant congenic strains of OcB/Dem series that carry identical *H2* locus. *Cent. Eur. J. Biol. 1: 16-28, 2006.*

4. Gusareva, E.S., Bragina, E.J., Deeva, E.V., Kazakevich N.V., Puzyrev, V.P., Ogorodova, L.M., Lipoldová M.: Cat is a major allergen in patients with asthma from west Siberia, Russia. *Allergy 61, 509-510, 2006.*

5. Havelková, H., Badalová, J., Svobodová, M., Vojtíšková, J., Kurey, I., Vladimirov, V., Demant, P., Lipoldová, M.: Genetics of susceptibility to leishmaniasis in mice: four novel loci and functional heterogeneity of gene effects. *Genes Immun., in press.*

6. Lipoldová, M., Demant, P.: Genetic susceptibility to infectious disease: lessons from mouse models of leishmaniasis. *Nat. Rev. Genet. 7: 294-305, 2006.*

Tumour Immunology

Head: Milan Reiniš

Research Fellows: Jan Bubeník, Jana Šímová, Marie Indrová, Táňa Jandlová, Romana Mikyšková (maternity leave)

Graduate Student: Jasper Manning Jr.

Undergraduate Students: Simona Moravcová, Tereza Böhmová, Ivan Štěpánek

Research Assistants: Jana Bieblová, Hana Přibylová

Technicians: Marie Malečková, Renata Turečková

Introduction

The long-term research programme of the laboratory is focused on investigation of the cell signals involved in induction and regulation of the anti-tumour immunity. The murine model for tumours associated with human papilloma virus (aetiologic agent of the cervix carcinoma) has been employed in most of our studies.

This principal model has been used for analysis of the missing signals and their delivery in the optimal form (e.g. by administration of recombinant cytokines, synthetic CpG oligodeoxynucleotides, antigen-loaded dendritic cells, or by gene therapy using vaccination with tumour cells carrying the inserted genes for the respective molecules) in order to induce or to restore the anti-tumour responses that can prevent or inhibit the growth of the transplanted tumours in experimental animals.

Special attention is paid to the clinically relevant setting of the minimal residual tumour disease treatment after primary tumour resection or chemotherapy. Using this model we investigate the mechanisms of immunosupression in the course of the tumour growth, with the final goal to include the blockage of the negative signals into the immunotherapeutic schemes.

Our effort is also focused on immunotherapy of the tumour cell variants that escaped the specific immune response mediated by cytotoxic T lymphocytes. Malignant conversion and subsequent *in vivo* selection can give rise to the cell populations that show stable expression of an immune escape phenotype, MHC class I deficient neoplasms. This makes the elucidation of the mechanisms of the effective anti-tumour responses against MHC class I-deficient tumours and development of immunotherapeutic protocols a very important task in tumour immunology. We have investigated the mechanisms of the anti-tumour immune responses against tumours mediated by MHC class I-restricted and -unrestricted mechanisms, immunologic cross-reactivity between tumours of the same aetiology but distinct MHC class I expression, the dynamics of MHC class I expression changes on tumour cells during the course of the tumour growth, and the impact of epigenetic modifications on the MHC class I expression.

Results

As an experimental model for tumour immunotherapy and gene therapy, murine carcinomas obtained after transfection of murine cells with plasmid DNA coding for E6/E7 HPV16 and activated Ha-ras oncogenes were utilized. The murine HPV 16-associated carcinoma system devel-

oped for the studies is mimicking human HPV16-associated carcinomas with regard to the aetiology, ability to metastasize and absence, presence or down-modulation of MHC class I molecules on the surface of tumour cells. In this sense, this experimental system is unique and extremely valuable for the development of therapeutic vaccines, particularly against MHC class I-negative HPV16-associated tumours, comprising approximately 55% of human cervical carcinomas.

Our previous studies indicated that particularly non-generalized early forms of cancer, small primary tumours, and especially minimal residual tumour disease induced by cytoreductive chemotherapy or surgery, and micrometastases should be considered for the immunotherapy of cancer. Local recurrences at the site of tumour resection as well as distant micrometastases manifested after surgery represent major problems in oncology. Adjuvant immunotherapy and gene therapy may help to cope, at least partially, with these problems. In the recent experiments, we have obtained priority data using the model of the minimal residual tumour disease and the tumours with down-regulated MHC class I expression.

Immunotherapy of MHC class I-positive and -deficient tumours with the synthetic unmethylated CpG oligodeoxynucleotides. Unmethylated oligodeoxynucleotides containing guanine-cytidine dimers (CpG ODN) have been described as potent inducers of antitumour immune responses. We hypothesized that CpG ODN therapy could be an effective tool for immunotherapy of not only conventional MHC class I^+ tumours, but also of those tumours that have lost MHC class I expression during their progression. To address this hypothesis, we employed our animal model resembling MHC class I-proficient and -deficient human papilloma virus (HPV) 16-associated tumours (MHC class I^+ TC-1, MHC class I^- TC-1/A9 variant and TC-1 P3C10 with down-modulated MHC class I expression). The therapy started either one day after the challenge with the tumour cells or later, when the tumours had reached a palpable size. In both settings, CpG ODN 1826 significantly reduced the growth of MHC class I-proficient and -deficient tumours. Furthermore, we demonstrated that CpG ODN 1585, whose mechanism of action preferably involves indirect activation of the natural killer cells, induced regression of the MHC class I-deficient tumours TC1/A9 but not of the MHC class I-proficient tumours TC-1. Moreover, the growth of recurrences after surgical removal of the primary tumours can be effectively inhibited. This study infers that synthetic CpG ODN have a potential for the therapy of tumours that tend to downregulate their MHC class I expression.

Therapy of HPV16-associated tumours disease with dendritic cell-based vaccines. Dendritic cell (DC)-based vaccines are being intensively investigated for the treatment of a variety of human neoplasms. However, little attention has until now been paid to the use of DC-based vaccines for immunotherapy of tumour residua after surgery. Therefore, we employed the animal model mimicking human HPV16-associated neoplasms to examine the effect of DC-based vaccines for the treatment of minimal residual tumour disease after surgery. Mice were subcutaneously inoculated with syngeneic TC-1 tumour cells. When the tumours reached approximately 1 cm in diameter, they were surgically removed and the operated mice were injected into the site of the operation with bone marrow-derived DC, which were either pulsed with TC-1 cell lysates or co-cultured with irradiated TC-1 cells. It has been found that the growth of TC-1 tumour recurrences in the mice treated with these vaccines was substantially suppressed, as compared to the operated-only controls.

In the next series of experiments we have tested the immunogenicity of dendritic cell-based

HPV16 E6/E7 peptide vaccines. We have investigated the capacity of cellular vaccines based on dendritic cells loaded with human HPV16 E6/E7 oncoprotein-derived peptides to induce immune responses *in vitro* and to elicit protective immunity. We have shown that the strongest protective effect against TC-1 tumours was achieved with the DC loaded with the peptide E7(44-62) harbouring both CTL and Th epitopes as compared to the shorter peptides containing only single CTL epitopes (e.g.E7 49-57). We have also examined the therapeutic efficacy of vaccines generated by fusion of HPV16-associated tumour cells TC-1 with syngeneic and allogeneic dendritic cells. Locally administered hybrid cells generated by fusion of MHC class I^+ TC-1 cells and syngeneic DC inhibited the growth of MHC class I^+ TC-1 tumours, but not the growth of MHC class I^- TC-1/A9-derived tumours.

MHC class I expression changes on the tumour cells during progression and therapy. Experiments were designed to examine whether the immune escape phenotype of HPV16-associated tumours is stable or whether the MHC class I expression can change during tumour progression and therapy. It has been found that temporary growth of HPV16-associated MHC class I-deficient tumours MK16/1/IIIABC in syngeneic mice can lead to upregulation of the low MHC class I expression, both in the subcutaneous tumour inocula and in their lung metastases. Mimicking this process *in vitro* by co-cultivation of tumour and spleen cell populations revealed that the spleen cells produce IFN , which upregulates MHC class I expression on the MK16/1/IIIABC cells and their sensitivity to T cell-mediated cytolysis (CTLs). Similar upregulation of the MHC class I expression was observed in other HPV16-associated tumour cell lines, MK16/MET/M1, TC-1, TC-1/A9 and TC-1/P3C10 grown *in vitro* in the presence of IFN . After removal of the IFN from the cultivation medium or after injection of the IFN -treated cells into syngeneic mice the MHC class I expression gradually dropped back to the original level or to the level observed in the tumours growing *in vivo*. Similarly, substantial upregulation of the MHC class I expression was observed in TC-1/A9 tumour recurrences after surgery. These findings indicate that the immune escape phenotypes of at least some HPV16-associated tumours are not stable and that upregulation of the MHC class I expression can occur *in vivo* during progression of the MHC I^- tumours, apparently due to production of IFN by the immune cells in the tumour microenviroment and its vicinity. If confirmed also with other tumour types and in human tumour systems, the upregulation of MHC class I molecule expression during radiotherapy and in tumour recurrences after surgery may have important implications in the development of immunotherapeutic strategies.

Chemotherapy, IL-12 gene therapy and combined adjuvant therapy of HPV16-associated MHC class I-proficient and -deficient tumours. Tumour cell line TC-1 (MHC class I^+) and its variants, TC-1/P3C10 and TC-1/A9, with a marked downregulation of MHC I molecules, were used to examine the effect of local interleukin 12 (IL-12) gene therapy in the treatment of early tumour transplants and minimal residual tumour disease obtained after cytoreductive chemotherapy (CMRTD). Experiments were designed to examine whether the downregulation of MHC class I molecules plays a role during chemotherapy and gene therapy of early tumour transplants. It was found that peritumoral administration of IL-12-producing tumour cell vaccines inhibited the growth of both TC-1 tumours and their MHC class I-deficient variants. Moreover, IL-12 gene therapy was utilized for the treatment of minimal residual tumour disease after cytoreductive chemotherapy. Intra-peritoneal treatment of tumour-bearing mice with ifosfamide derivative, CBM-4A, produced a significant tu-

mour-inhibitory effect. This treatment was followed by peritumoral s.c. administration of genetically modified TC-1 or MK16/I/IIIABC (MHC class I^-) vaccines producing IL-12 or with recombinant interleukin 12 (rIL-12). This combined therapy significantly inhibited the growth of TC-1 and TC-1/A9 tumours. The impacts of chemotherapy and IL-12 adjuvant therapy on MHC class I surface expression were assessed. Chemotherapy and gene therapy of tumours led to the upregulation of MHC I expression on MHC class I-deficient tumours (TC-1/A9 and TC-1/P3C10) and to downregulation on MHC I-proficient tumours (TC-1). These findings indicate that the MHC I phenotype is not stable during tumour progression and treatment.

Our projects were supported by grants Nos. NC/7148-3, NR/8004-3 and NR/7807-3 from the Grant Agency of the Ministry of Health of the Czech Republic, Nos. 301/01/0985, 301/04/1492 and 310/05/H533 from the Grant Agency of the Czech Republic; by projects Nos. K5011112 and A5052203 awarded by the Academy of Sciences of the Czech Republic, by NIH Research Grant No. D43 TW000233 funded by the Fogarty International Center, the National Institute on Drug Abuse and the National Heart Lung and Blood Institute, and by the League against Cancer, Prague.

Publications

1. Mikyšková, R., Indrová, M., Šímová, J., Jandlová, T., Bieblová, J., Jinoch, P., Bubeník, J., Vonka, V.: Treatment of minimal residual tumour disease after surgery or chemotherapy in mice carrying HPV16-associated tumours: cytokine and gene therapy with IL-2 and GM-CSF. *Int. J. Oncol. 24: 161-168, 2004.*

2. Bubeník, J.: MHC class I down-regulation: Tumour escape from immune surveillance? *Int. J. Oncol. 25: 487-491, 2004.*

3. Bubeník, J.: Interleukin-2 therapy of cancer. *Folia Biol. (Praha) 50: 120-130, 2004.*

4. Reiniš, M., Indrová, M., Mendoza, L., Mikyšková, R., Bieblová, J., Bubeník, J., Šímová, J.: HPV 16-associated tumours: Therapy of surgical minimal residual disease with dendritic cell-based vaccines. *Int. J. Oncol. 25: 1165-1170, 2004.*

5. Indrová, M., Reiniš, M., Bubeník, J., Jandlová, T., Bieblová, J.: Immunogenicity of dendritic cell-based HPV 16 E6/E7 peptide vaccines: CTL activation and protective effects. *Folia Biol. (Praha) 50: 184-193, 2004.*

6. Mikyšková, R., Bubeník, J., Vonka, V., Šmahel, M., Indrová, M., Bieblová, J., Šímová, J., Jandlová, T.: Immune escape phenotype of HPV16-associated tumours: MHC class I expression changes during progression and therapy. *Int. J. Oncol. 26: 521-527, 2005.*

7. Bubeník, J., Šímová, J.: Minimal residual disease as the target for immunotherapy and gene therapy of cancer (review). *Oncol. Rep. 14: 1377-1380, 2005.*

8. Šímová, J., Bubeník, J., Bieblova, J., Indrová, M., Jandlová, T.: Immunotherapeutic efficacy of vaccines generated by fusion of dendritic cells and HPV16-associated tumour cells. *Folia Biol. (Praha) 51: 19-24, 2005.*

9. Indrová, M., Bieblová, J., Jandlová, T., Vonka, V., Pajtasz-Piasecka, E., Reiniš, M.: Chemotherapy, IL-12 gene therapy and combined adjuvant therapy of HPV16-associated MHC class I-proficient and -deficient tumours. *Int. J. Oncol. 28: 253-259, 2006.*

10. Reiniš, M., Šímová, J., Bubeník, J.: Inhibitory effects of unmethylated CpG oligodeoxynucleotides on MHC class I-deficient and -proficient HPV16-associated tumours. *Int. J. Cancer 118: 1836-1842, 2006.*

11. Chakraborty R, Reinis M., Rostron T., Philpott S., Dong T., D'Agostino A., Musoke R., de Silva E., Stumpf M., Weiser B., Burger H., Rowland-Jones S.L.: nef gene sequence variation among HIV-1- infected African children. *HIV Med. 7: 75-84, 2006.*

Transplantation Immunology

Head: Alena Zajícová

Research Fellows: Vladimír Holáň, Magdaléna Krulová

Graduate Students: Jan Frič[1], Jana Pindjáková[1] , Andrea Vítová[2], Kateřina Pokorná[1], Aleš Neuwirth[1]

Undergraduate Student: Jana Procházková

Technicians: Lucie Holáňová, Jaroslava Knížová

[1] Supported by Faculty of Science, Charles University, Prague
[2] Supported by 1st Medical Faculty, Charles University, Prague

Introduction

Immunological rejection represents the major obstacle for further development of clinical transplantation**.** Therefore, the insight into the cellular and molecular mechanisms of immunological reaction and possibility to manipulate with the immune response is the major task of our research group. The research is focused on elucidation and characterization of the role of individual cell subpopulations, cytokines and other immunologically relevant molecules in the modulation of specific immunity. For these purposes, a large scale of *in vivo* and *in vitro* techniques of cellular and molecular immunology have been established.

As basic *in vivo* models we use the model of skin grafting and the technique of orthotopic corneal transplantation in laboratory animals. The first model represents grafting into a conventional site, whereas the second model represents grafting into an immunologically privileged site. Using *in vitro* models of transplantation reaction, such as mixed lymphocyte culture or cytotoxicity tests, molecular mechanisms of the immune reaction were tested. The research was focused on characterization of the regulatory role of individual interleukins and other cytokines and on their effects on the expression of genes for other molecules involved in the immunity. The models of allotransplantation, which were studied originally, were recently extended to the xenotransplantation system. For the future, xenotransplantations can overcome the growing shortage of the allografts for clinical transplantations.

On the basis of our results, new approaches for immunoregulation are being proposed and tested. The ultimate goal of our research is to get insight into the mechanisms of specific immune response and to propose and test novel strategies to increase or suppress immune responses in a desirable manner.

Results

In our previous studies we have demonstrated that nitric oxide (NO) produced by allograft-infiltrating macrophages represents an important effector molecule contributing to the damage of the grafted tissue. However, contrary to the rejection of allografts, NO generation was not found in rejected skin xenografts, in spite of an abundant expression of mRNA for inducible NO synthase (iNOS) and accumulation of iNOS protein (10). We proved that the absence of NO formation during xenograft rejection is due to the activi-

ty of arginase, which is synthesized in a significant amount during xenograft rejection (14). We demonstrated that Th2 cytokines, which are predominantly produced after xenotransplantation, stimulate expression of genes for arginase I and arginase II and a high arginase activity can be found in rejected xenografts. The arginase successfully competes with iNOS for L-arginine, which is a common substrate for iNOS and arginase. As a result, the availability of L-arginine for iNOS becomes limited and NO generation is attenuated. We showed that suppression of arginase activity with specific arginase inhibitors restored NO production in xenografts (14). These results altogether demonstrated that the balance in Th1/Th2 cytokines after transplantation regulates the ratio arginase/iNOS in the graft and that these enzymes regulate the effector mechanisms of transplantation reactions.

Our well-established models of corneal transplantation enable us to demonstrate that monoclonal antibodies against anti-$CD4^+$ cells are the most effective agents in obtaining prolonged survival or even tolerance of both corneal allografts (2) and xenografts (12). Other studies on the model of corneal transplantation have shown distinct roles for various draining lymph nodes in induction of corneal graft survival (4), and we also demonstrated an important role of corneal endothelium in susceptibility of corneal grafts to immunological attack (3). By adoptive transfers of the immune serum we showed for the first time that corneal grafts are vulnerable to the effects of cytotoxic antibodies (11). Studies of specific features of immunity in the eye resulted in a description of a new factor that is produced by corneal stromal cells and that selectively inhibits production of anti-inflammatory cytokines by activated T cells (1).

Well-established methods for detection of cytokines and for evaluation of their immunoregulatory role enabled us to characterize the immunomodulatory effects of boar seminal immunosuppressive fraction (9), various psychostimulants (6), opiates in experimental models (7) or in heroin addicts and patients maintained on methadone (5), to test changes in the immunological reactivity of mice treated for cancer (8) or to characterize the immunoregulatory properties of novel model particles produced on the basis of polyoma virus capsids (13).

The work of the group was supported by grant No. 310/02/D162 from the Grant Agency of the Czech Republic, grants Nos. NR/7816-3, NR/8340-3 and NI/7531-3 from the Grant Agency of the Ministry of Health of the Czech Republic, the projects LN00A026 and 1M6837805001 from the Ministry of Education, Youth and Sports of the Czech Republic, and by grant No. 018094 from the European Union.

Publications

1. Holáň, V., Vítová, A., Pindjáková, J., Krulová, M., Zajícová, A., Filipec, M.: Corneal stromal cells selectively inhibit production of anti-inflammatory cytokines by activated T cells. *Clin. Exp. Immunol. 136: 200-206, 2004.*

2. Vítová, A., Filipec, M., Zajícová, A., Krulová, M., Holáň, V.: Prevention of corneal allograft rejection in a mouse model of high-risk recipients. *Br. J. Ophthalmol. 88: 1338-1342, 2004.*

3. Plšková, J., Kuffová, L., Filipec, M., Holáň, V., Forrester, J. V.: Quantitative evaluation of the corneal endothelium in the mouse after grafting. *Br. J. Ophthalmol. 88: 1209-1216, 2004.*

4. Plšková, J., Holáň, V., Filipec, M., Forrester, J. V.: Lymph node removal enhances corneal graft survival in mice at high-risk of rejection. *MRC Ophthalmol. 4: 3-9, 2004.*

5. Zajícová, A., Wilczek, H., Holáň, V.: The alterations of immunological reactivity in heroin addicts and their normalization in patients maintained on methadone. *Folia Biol. (Praha) 50: 24-28, 2004.*

6. Basta-Kaim, A., Budziszewska, B., Jaworska-Feil, L., Tetich, M., Kubera, M., Zajícová, A., Holáň, V., Lason, W.: Effects of lipopolysaccharide and chlorpromazine on glucocorticoid receptor-mediated gene transcription and immunoreactivity – a possible involvement of p38 kinase. *Eur. Neuropsychopharmacol. 14: 521-528, 2004.*

7. Kubera, M., Filip, M., Basta-Kaim, A., Siwanowicz, J., Nowak, E., Zajícová, A., Holáň, V., Budziszewska, B., Maes, M., Lason, W.: The effect of cocaine sensitization on mouse immunoreactivity. *Eur. J. Pharmacol. 483: 309-315, 2004.*

8. Sobotková, E., Dušková, M., Šmahel, M., Holáň, V., Janoušková, O., Vonka, V.: Chemotherapy and immunotherapy of tumours induced by gene-modified HPV16-transformed cells. *Oncol. Rep. 12: 877-883, 2004.*

9. Veselský, L., Holáň, V., Dostál, J., Železná, B.: Boar seminal immunosuppressive fraction attenuates the leptin concentration and restores the thymus mass during pregnancy in mice. *Reproduction 127: 581-585, 2004.*

10. Holáň, V., Pindjáková, J., Zajícová, A., Krulová, M., Železná, B., Matoušek, P., Svoboda, P.: The activity of inducible nitric oxide synthase in rejected skin xenografts is selectively inhibited by a factor produced by grafted cells. *Xenotransplantation 12: 227-234, 2005.*

11. Holáň, V., Vítová, A., Krulová, M., Zajícová, A., Neuwirth, A., Filipec, M., Forrester, J.V.: Susceptibility of corneal allografts and xenografts to antibody-mediated rejection. *Immunol. Lett. 100: 211-213, 2005.*

12. Pindjáková, J., Vítová, A., Krulová, M., Zajícová, A., Filipec, M., Holáň, V.: Corneal rat-to-mouse xenotransplantation and the effects of anti-CD4 or anti-CD8 treatment on cytokine and nitric oxide production. *Transplant Int. 18: 854-862, 2005.*

13. Bouřa, E., Liebl, D., Spíšek, R., Frič, J., Marek, M., Štokrová, J., Holáň, V., Forstová, J.: Polyomavirus EGPF-pseudocapsids: Analysis of model particles for introduction of proteins and peptides into mammalian cells. *FEBS Lett. 579: 6549-6558, 2005.*

14. Holáň, V., Pindjáková, J., Krulová, M., Neuwirth, A., Zajícová, A., Frič, J.: Production of niric oxide during graft rejection is regulated by the Th1/Th2 balance, the arginase activity and L-arginine metabolism. *Transplantation, in press.*

Genomics and Bioinformatics

Head: Čestmír Vlček

Research Fellows: Jan Pačes, Pavel Urbánek (from March 2005)

Graduate Students: Zdeněk Krejčík, Hynek Strnad, Radek Zika, Zdeněk Chodora, Miluše Hroudová

Technician: Šárka Pinkasová

Associated with the group: Václav Pačes

Introduction

Genomics and bioinformatics belong to the most vigorously developing disciplines of contemporary life sciences. The Department of Genomics and Bioinformatics was among the first laboratories to complete a genome project. It was in the mid eighties that the complete nucleotide sequence of the bacteriophage PZA DNA was determined. Since then the laboratory has been developing new approaches and strategies to the large-scale DNA sequencing and was involved in several international genome projects such as the Pseudorabies virus and bovine herpesvirus genome projects, *Saccharomyces cerevisiae* genome project and *Rhodobacter capsulatus* genome project. Information generated in these projects was used in evolutionary studies and recently also in biotechnological applications. In collaboration with other groups of the Institute, individual members of the Department became involved in characterization of several developmental genes and in studies of the transcriptional profiles of expression of several mouse genes. With the advances in the human genome project and mouse genome project we now analyse these genomes especially as concerns foreign elements present in these and other (e.g. bacterial) genomes.

The tools of bioinformatics were developed in order to analyse nucleotide sequences generated in these projects as well as those available in the international databases.

Results

The international genome project of *Rhodobacter capsulatus* has been completed (1). Several genes with possible biotechnology applications are now being characterized.

Special attention was devoted to human genome analysis, namely to the isochore structures of individual human chromosomes. In addition, distribution of Alu and LINE repetitive sequences was characterized. HERVd, the database of human endogenous retroviruses, was assembled (2). This database is now available at http://herv.img.cas.cz. It is being used for detailed characterization of HERVs, their integration, stability, and distribution (3,4).

In collaboration with botanists, phylogenetic analyses based on chloroplast, rRNA, and nuclear DNA sequences were performed for several plant types (5-9).

Starting from March 2005, the Laboratory of Developmental Molecular Genetics (headed by P. Urbánek) joined the Department, focusing on the function and comparative genomics of developmental gene families. We characterized the predicted repressed gene of *Arabidopsis thaliana* (At4g21130) that codes for a homologue of the U3-55K proteins from *Arabidopsis*, man, mouse, yeast and other eukaryotes. In man and yeast, U3-55K is involved in the processing of the preribosomal RNA. We showed that treatment with inhibitors of histone deacetylases or DNA meth-

yltransferases induces a low but distinct level of mRNA from the repressed *Arabidopsis* gene. Sequencing of RT-PCR products revealed the open reading frame that differs, in part, from the hypothetical one and encodes a seven-WD-repeat protein highly similar to U3-55K proteins from diverse eukaryotic species. The described approach may help determine the nucleotide sequences of transcripts from predicted genes with a low level of expression (10).

The sequencing of the mouse testis SAGE library was finished and the testes transcriptome was described (11).

Publications

1. Haselkorn, R., Lapidus, A., Kogan, Y., Vlček, Č., Pačes, J., Pačes, V., Ulbrich, P., Pečenková, T., Rebrekov, D., Milgram, A., Mazur, M., Cox, R., Kyrpides, N., Ivanova, N., Kapatral, V., Los, T., Lykidis, A., Mikhailova, N., Reznik, G., Vasieva, O., Fonstein, M.: The *Rhodobacter capsulatus* genome. *Photosynthesis Res. 70: 43-52, 2001.*

2. Pačes, J., Pavlíček, A., Zíka, R., Kapitonov, V. V., Jurka, J., Pačes, V.: HERVd: the Human Endogenous Retrovirus Database: update. *Nucleic Acids Res. 32: 50, 2004.*

3. Belshaw, R., Katzourakis, A., Pačes, J., Burt, A., Tristem, M.: High copy number in human endogenous retrovirus families is associated with copying mechanisms in addition to reinfection. *Mol. Biol. Evol. 22: 814-817, 2005.*

4. Pavlíček, A. Gentles, A.J., Pačes, J., Pačes, V., Jurka, J.: Retroposition of processed pseudogenes: the impact of RNA stability and translational control. *Trends Genet. 22: 69-73, 2006.*

5. Drábková, L., Kirschner, J., Seberg, O., Petersen, G., Vlček, Č.: Phylogeny of the Juncaceae based on rbcL sequences, with special emphasis on Luzula DC. and Juncus L. *Plant Systemat. Evol. 240: 133-147, 2003.*

6. Drábková, L., Kirschner, J., Vlček, Č., Seberg, O., Petersen, G.: A phylogeny of Luzula and Juncus (Juncaceae) based on rbcL, trnL intron, and trnL-trnF plastid DNA sequences. *Cladistics 19: 151-151, 2003.*

7. Drábková, L., Kirschner, J., Vlček, Č., Pačes, V.: TrnL-trnF intergenic spacer and trnL intron define clades within Luzula and Juncus (Juncaceae). *J. Mol. Evol. 59: 1-10, 2004.*

8. Drábková, L., Kirschner, J., Vlček, Č.: Phylogenetic relationships within Luzula DC. and Juncus L. (Juncaceae): a comparison of phylogenetic signal of trnL-F intergenic spacer, trnL intron and rbcL plastome sequence data. *Cladistics, in press.*

9. Drábková, L., Vlček, Č.: The phylogenetic position of Oxychloe (Juncaceae): evidence from one nuclear, three plastid regions and morphology. *Taxon, in press.*

10. Urbánek, P., Pačes, J., Pačes, V.: An approach towards experimental cDNA sequence determination of predicted genes: An example from *Arabidopsis* U3-55k homologues. *Gene 358: 67-72, 2005.*

11. Divina, P., Vlček, Č., Strnad, P., Pačes, V., Forejt, J.: Global transcriptome analysis of the C57BL/6J mouse testis by SAGE: evidence for nonrandom gene order. *BMC Genomics 6: 29, 1-17, 2005.*

Mammalian Molecular Genetics

Head: Jiří Forejt

Research Fellows: Soňa Gregorová, Petr Jansa, Zdeněk Trachtulec

Graduate Students: Petr Divina, Robert Ivánek, Ondra Mihola, Radka Storchová, Tomáš Vacík (until December 2004), David Homolka (since October 2005), Radek Blatný (since June 2005)

Technicians: Marta Landíková, Vladana Fotopulosová

Introduction

Genomes of most classical laboratory mouse inbred strains originated predominantly from the *Mus musculus domesticus* subspecies and in about 20 % from a related *Mus musculus musculus* subspecies. Since both subspecies have evolved independently for at least 350 000 years, they accumulated a high number of SNPs (single nucleotide polymorphisms) in their genomes and a high number of heritable phenotypic variants. Thus, genetic analyses of classical laboratory strains and strains derived from *M. m. musculus* are likely to be highly informative for functional genomics studies (2).

The group of Mammalian Molecular Genetics is using *M. m. musculus*-derived mouse inbred strain PWD/Ph to develop a set of 21 chromosome substitution strains with individual chromosomes of PWD origin on the genetic background of the B6 strain. The main research topics of the group further include segmental trisomy Ts43H as a mouse model of human aneuploidy syndromes. Mapping and positional cloning of mouse hybrid sterility genes belong to the long-term goals of the group.

Results

The construction of inter-subspecies mouse chromosome substitution strains was almost finished in the fifth year of the project. By December 2005, 21 consomic or subconsomic strains were completed and 8 remaining strains will be finished in 2006. Over 88 000 genotypes have been analysed so far using 315 SSLP markers. The first genomic BAC library from the *Mus m. musculus* mice (PWD/Ph) was prepared and characterized (11). It will become publicly available via RZPD.

Segmental trisomy of the proximal part of Chr 17, Ts43H, has been characterized as a mouse model of human aneuploidy syndromes (9). A new hybrid sterility gene, *Hstx1*, has been mapped on Chromosome X (4). Positional cloning of the Hybrid sterility 1 (*Hst1*) gene continued by narrowing down the critical region from 540 to 360 kilobases. Two out of six potential candidate genes, namely TATA binding protein (*Tbp*) and proteasome subunit beta 1 (*Psmb1*) were sequenced and their expression analysed in sterile and fertile testis. None of them seem to be identical with *Hst1* (3,7,8). The Mouse SAGE Site database of publicly available SAGE libraries generated from mouse tissues and cell lines was constructed (1). Currently, the database contains data from 65 mouse SAGE libraries, including one prepared from adult C57BL/6J testis (10), and provides easy-to-use tools for browsing, comparing and searching the SAGE data. The Mouse SAGE Site is freely accessible on the Internet address http://mouse.biomed.cas.cz/sage/ (1).

The work of the group was supported by the Grant Agency of the Czech Republic (grant No. 301/05/0738), by the Grant Agency of the Academy of Sciences of the Czech Republic (grant No. A5052406), by the Czech Ministry of Education, Youth and Sports (project No. LN00A079 - Center of Integrated Genomics), by NIH (grant No. 1 R01 HG003183-01) and by Howard Hughes Medical Institute.

Publications

1. Divina, P., Forejt, J.: The mouse SAGE site: database of public mouse SAGE libraries. *Nucleic Acids Res. 32: D482-D483, 2004.*

2. Fernandes, C., Liu, L., Paya-Cano, J.L, Gregorova, S., Forejt, J., Schalkwyk, L.J.: Behavioural characterisation of wild derived male mice (Mus musculus musculus) of the PWD/Ph inbred strain: high exploration compared to C57BL/6J. *Behav. Genet. 34: 621-630, 2004.*

3. Trachtulec, Z., Vlček, C., Mihola, O., Forejt, J.: Comparative analysis of the PDCD2-TBP-PSMB1 region in chordates. *Gene 335: 151-157, 2004.*

4. Storchová, R., Gregorová, S., Buckiová, D., Kyselová, V., Divina, P., Forejt, J.: Genetic analysis of X-linked hybrid sterility in the house mouse. *Mamm. Genome 15: 515-524, 2004.*

5. Churchill, G.A., Airey, D.C., Allayee, H., Angel, J.M., Attie, A.D., Beatty, J., Beavis, W.D., Belknap, J.K., Bennett, B., Berrettini, W., Bleich, A., Bogue, M., Broman, K.W., Buck, K.J., Buckler, E., Burmeister, M., Chesler, E.J., Cheverud, J.M., Clapcote, S., Cook, M.N., Cox, R.D., Crabbe, J.C., Crusio, W.E., Darvasi, A., Deschepper, C.F., Doerge, R.W., Forejt, J., Gu, J., Gu, W., de Haan, G., Hayes, N.L., Heller, C., Himmelbauer, H., Hitzemann, R., Hunter, K., Hsu, H.C., Iraqi, F.A., Ivandic, B., Jacob, H.J., Jansen, R.C., Jepsen, K.J., Johnson, D.K., Johnson, T.E., Kempermann, G., Kendziorski, C., Kotb, M., Kooy, R.F., Llamas, B., Lammert, F., Lassalle, J.M., Lowenstein, P.R., Lu, L., Lusis, A., Manly, K.F., Marcucio, R., Matthews, D., Medrano, J.F., Miller, D.R., Mittleman, G., Mock, B.A., Mogil, J.S., Montagutelli, X., Morahan, G., Morris, D.G., Mott, R., Nadeau, J.H., Nagase, H., Nowakowski, R.S., O'Hara, B.F., Osadchuk, A.V., Page, G.P., Paigen, B., Paigen, K., Palmer, A.A., Pan, H.J., Peltonen-Palotie, L., Peirce, J., Pomp, D., Pravenec, M., Prows, D.R., Qi, Z., Reeves, R.H., Roder, J., Rosen, G.D., Schadt, E.E., Schalkwyk, L.C., Seltzer, Z., Shimomura, K., Shou, S., Sillanpaa, M.J., Siracusa, L.D., Snoeck, H.W., Spearow, J.L., Svenson, K., Tarantino, L.M., Threadgill, D., Toth, L.A., Valdar, W., de Villena, F.P., Warden, C., Whatley, S., Williams, R.W., Wiltshire, T., Yi, N., Zhang, D., Zhang, M., Zou, F.; Complex Trait Consortium: The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat. Genet. 36: 1133-1137, 2004.*

6. Auwerx, J., Avner, P., Baldock, R., Ballabio, A., Balling, R., Barbacid, M., Berns, A., Bradley, A., Brown, S., Carmeliet, P., Chambon, P., Cox, R, Davidson, D., Davies, K., Duboule, D., Forejt, J., Granucci, F., Hastie, N., de Angelis, M.H., Jackson, I., Kioussis, D., Kollias, G., Lathrop, M., Lendahl, U., Malumbres, M., von Melchner, H., Muller, W., Partanen, J., Ricciardi-Castagnoli, P.,

Rigby, P., Rosen, B., Rosenthal, N., Skarnes, B., Stewart, A.F., Thornton, J., Tocchini-Valentini, G., Wagner, E., Wahli, W., Wurst, W.: The European dimension for the mouse genome mutagenesis program. *Nat. Genet. 36: 925-927, 2004.*

7. Trachtulec, Z., Mihola, O., Vlček, C., Himmelbauer, H., Pačes, V., Forejt, J.: Positional cloning of the Hybrid sterility 1 gene: fine genetic mapping and evaluation of two candidate genes. *Biol. J. Linn. Soc. 84: 637-641, 2005.*

8. Vyskočilová, M., Trachtulec, Z., Forejt, J., Pialek, J.: Does geography matter in the hybrid sterility in house mice? *Biol. J. Linn. Soc. 84: 663-674, 2005.*

9. Vacík, T., Ort, M., Gregorová, S., Strnad, P., Blatný, R., Conte, N., Bradley, A., Bureš, J., Forejt. J.: Segmental trisomy of Chromosome 17 – a new model of human aneuploidy syndromes. *Proc. Natl. Acad. Sci. USA 102: 4500-4505, 2005.*

10. Divina, P., Vlček, Č., Strnad, P., Pačes, V., Forejt, J.: Global transcriptome analysis of the C57BL/6J mouse testis by SAGE: evidence for nonrandom gene order. *BMC Genomics 6: 29, 2005.*

11. Jansa, P., Divina. P., Forejt, J.: Construction and characterization of a genomic BAC library for the Mus m. musculus mouse subspecies (PWD/Ph inbred strain). *BMC Genomics 6: 161, 2005.*

Cellular and Viral Genetics

Head: Jiří Hejnar

Research Fellows: Josef Geryk, Jiří Plachý, Jan Svoboda, Kateřina Trejbalová (from June 2005), Jan Závada, Zuzana Závadová

Graduate Students: Jana Blažková[1,2], Daniel Elleder (until October 2004), Magda Matoušková, Dana Průková[1], Volodymyr Stepanets, Filip Šenigl[1]

Research Assistants: Věra Hoserová, Markéta Reinišová (part time)

Undergraduate Students: Lubomíra Fričová, Jan Kotáb

Technicians: Helena Burešová (part time), Jitka Dvořáková (part time), Lenka Mikušová, Iva Rybková, Kamila Thunová

[1] Supported by Faculty of Science, Charles University, Prague
[2] Supported by the SIDAction

Introduction

Molecular interactions between invading retroviruses and infected host cells, the long-term interest of our group, are the central topics in virology nowadays. Deep insight into the virus-cell interplay not only helps in our understanding the viral pathogenesis but offers numerous targets for rational therapeutic intervention.

Retroviruses enter their natural host cells via specific receptors, i.e. cell surface transmembrane glycoprotein molecules that interact with trimers of viral surface (SU) glycoproteins and mediate fusion of viral and cellular membranes. The complex of avian sarcoma and leukosis viruses (ASLV) comprises at least five highly related envelope subgroups, which have been known to use various receptors from non-related protein families. Only two of these receptors have been identified up to now. So, in addition to their importance for studying the early events of retrovirus infection, ASLVs are an example of receptor usage evolution and broadening the infection capacity of the ancestral retrovirus. We have focused on the subgroup C viruses whose receptor has been enigmatic so far.

For the aforementioned project, we have broadly used our traditional models – RSV *in vitro* infection of avian vs mammalian cells or *in vivo* sarcoma induction in chickens. A substantial part of our research was based on our panel of inbred chicken lines, some of which are congenic in the major histocompatibility complex (MHC). We have currently improved our capacity in experimental poultry science by a new technology of knock-outing chicken genes in the pre-B cell line DT40 and by establishing a new approach of chicken transgenesis.

We have also continued our previous studies on human endogenous retroviruses (HERVs) and L1 retrotransposons. We have concentrated on the HERV-W family in which we have described the numerous processed pseudogenes created by the retrotransposition activity of L1s. The prototypic member of HERV-W family, ERVWE1, encodes the complete and functional viral transmembrane (TM) and SU proteins that serve for

cell-to-cell fusion in the syncytiotrophoblastic layer of human placenta. Expression of this captive retroviral envelope, *syncytin-1*, is tissue-specific and must be tightly regulated in non-placental tissues. We have analysed the epigenetic mechanisms involved in *syncytin-1* downregulation.

The last but not least research topic in our group has been characterization of the novel tumour-associated CA IX protein with carbonic anhydrase activity. Among 14 human carbonic anhydrases, only CA IX is strongly expressed in several types of cancer (renal, cervical, lung, colorectal, etc.) and, at the same time, it is absent in most of the normal tissues. A unique feature of CA IX is the presence of the proteoglycan-like domain (PG) located on its apical end. This suggests a possible relevance of PG in oncogenesis. Previously we demonstrated that PG mediates cell-to-cell adhesion, in which the repetitive motif PGEEDLP is involved (10).

Results

We have employed the growing capacity of chicken genomic tools for precise genetic **mapping of genes encoding receptors for ASLV-A and ASLV-C** on chicken chromosome 28 using two chicken inbred lines that carry different alleles coding for either resistance (Tvc^R) or sensitivity (Tvc^S) to infection by subgroup C viruses. A backcross population was tested for susceptibility to subgroup C infection and genotyped for markers from chicken chromosome 28. We have confirmed the close linkage between *Tva* and *Tvc* loci (1) and narrowed down the *Tvc* candidate region to the bacterial artificial chromosome (BAC)-mediated complementation screening in an effort to identify the unknown subgroup C receptor.

The BAC containing a portion of chicken chromosome 28 that conferred susceptibility to ASLV-C infection was found. The *Tvc* gene was identified on this genomic DNA fragment and encodes a 488-amino-acid protein most closely related to mammalian butyrophilins, members of the immunoglobulin superfamily (Fig. 1A). We have proved the *Tvc* identity by conferring the sensitivity to ASLV-C by *Tvc* cDNA (Fig. 1B) and by deletion of both *Tvc* alleles in the susceptible chicken DT40 cells. We have also identified a mutation in the *Tvc* gene in line L15 chickens that explains its resistance to ASLV-C infection (12).

Looking at the *Tva* sequences in inbred chicken lines, we have also found C40W mutation in the critical LDL-binding domain of this receptor in ALV-A-resistant chicken line C. The striking reduction of the ability to confer susceptibility to ALV-A as well as the decrease in binding affinity to the soluble ALV-A envelope glycoprotein have been demonstrated (2).

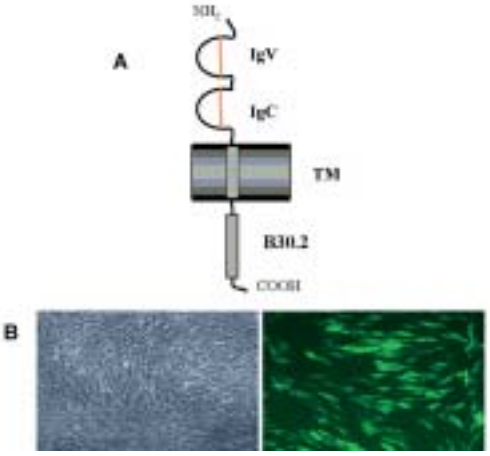


Fig. 1. Identification of the ASLV-C receptor, Tvc. (A) Schematic diagram of the Tvc protein with variable and constant Ig-like domains, IgV and IgC, transmembrane domain, TM, and butyrophilin domain, B30.2. (B) Expression of the *tvc* cDNA allows ASLV-C to infect resistant chicken cells. L15 chicken cells were transfected with *tvc* and infected with a GFP reporter virus. Images were taken with a phase contrast microscope (left) and with a fluorescence microscope (right).

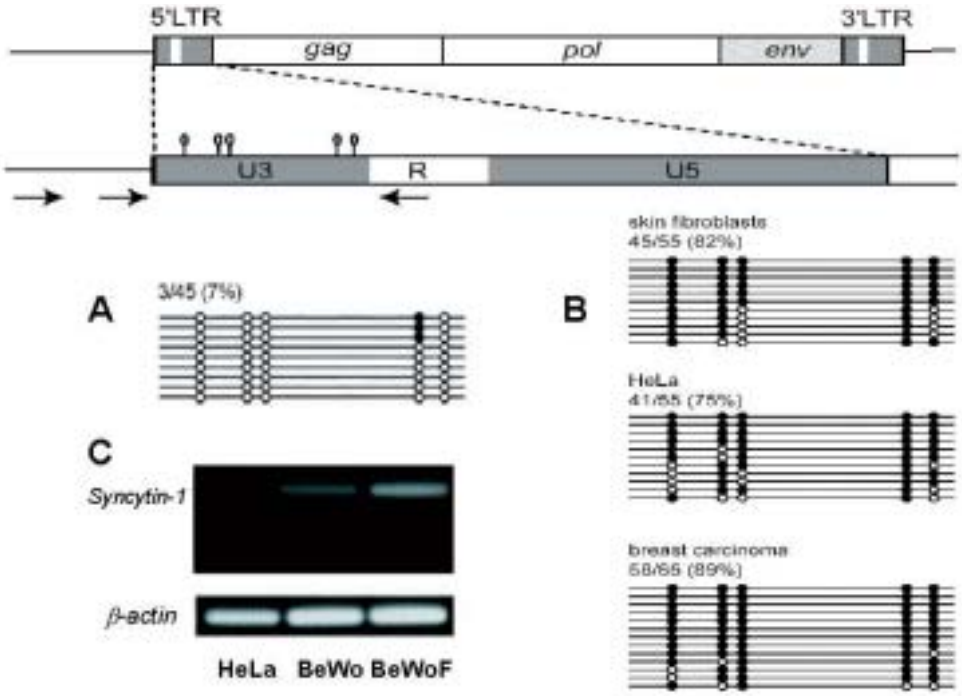


Fig. 2. CpG methylation status of the U3 region of ERVWE1 LTR as detected by bisulphite sequencing in (A) human choriocarcinoma BeWo cells and (B) non-placental cells or tumours. Methylated CpG dinucleotides are depicted by black circles, unmethylated CpGs indicated by open circles. The percentage of methylated CpGs is given in the parenthesis. (C) Expression of *syncytin-1* in HeLa cells, BeWo cells and syncytium-forming BeWo cells induced by forskolin treatment.

In addition to the avian exogenous retroviruses, we are also interested in **CpG methylation of HERV-Ws, especially the *syncytin-1***, a captive HERV whose functional *env* is used in the process of syncytiotrophoblast differentiation. Studying the CpG methylation patterns of the *syncytin-1* long terminal repeat (LTR), we have described an inverse correlation between LTR methylation and *syncytin-1* expression. Hypomethylation of the *syncytin-1* LTR in the placenta and in the choriocarcinoma-derived cell line BeWo (Fig. 2A), in contrast to heavily methylated *syncytin-1* LTR in non-placental tissues (Fig. 2B), suggests that transcriptional suppression of this gene is mediated in an epigenetic way and that demethylation of the LTR sequence is a prerequisite for its expression (Fig. 2C) and differentiation of multinucleated human syncytiotrophoblast (13).

We have examined the **chicken *TP53* tumor suppressor gene** in v-*src*-transformed chicken tumour cell lines. Using RT-PCR and DNA sequencing, we have detected frequent deletions of variable length in both DNA-binding and oligomerization domains of the *TP53*. The same pattern of *TP53* deletions was found in late as well as early *in vitro* passages of one chicken tumour cell line, PR9692 (6). This tumour cell line shows an immortal phenotype and acquires a metastatic potential that is unique in our experimental model of v-*src*-induced tumours in congenic chickens.

Further characterization of the PG domain of CA IX carbonic anhydrase revealed the following news (11): a remarkable feature of PG is a high prevalence of dicarboxylic amino acids, resulting in the acidic character of PG. This is reflected by pH dependence of binding between PG and specific antibody and between PG and its putative cell surface receptor. We also tested a series of human and animal cells for adhesion to immobilized human CA IX. In contrast to most human and rat cell lines, mouse cells do not attach to human CA IX because of the different PG sequence. PG is highly conserved in human, rat and even chicken, but not in the mouse orthologue Car IX. The result of our cell attachment test suggests co-variation of CA IX-PG and its putative receptor (11). At present, we are attempting to identify the putative PG-binding cell surface molecule in order to explain the role of CA IX in oncogenesis.

The members of the Cellular and Viral Genetics group also participated as collaborators in other projects on the database of HERVs (3), new tools of multiple sequence alignment (4), human genome isochore composition (5), development of a chick-embryo model for screening anti-metastatic drugs (7), the role of CCR2 chemokine receptor and $CCR2^{+}$ T cells during the inflammatory response in mice (8), the creation of a chicken DT40 bursal cell EST database (9), chicken transgenesis (14), and mapping the common retroviral integration sites in avian nephroblastoma (15).

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 204/01/0632, and 524/01/0866), Grant Agency of the Academy of Sciences of the Czech Republic (research grant A5052207), Ministry of Agriculture of the Czech Republic (research grant QE1001/2001/01), Bayer Corporation, Austrian Ministry of Science and Research, and INSERM.

Publications

1. Elleder, D., Plachý, J., Hejnar, J., Geryk, J., Svoboda, J.: Close linkage of genes encoding receptors for subgroups A and C of avian sarcoma/leucosis virus on chicken chromosome 28. *Anim Genet. 35: 176-181, 2004.*

2. Elleder, D., Melder, D.C., Trejbalová, K., Svoboda, J., Federspiel, M.J.: Two different molecular defects in the Tva receptor gene explain the resistance of two tvar lines of chickens to infection by subgroup A avian sarcoma and leukosis viruses. *J. Virol. 78: 13489-13500, 2004.*

3. Pačes, J., Pavlíček, A., Zíka, R., Kapitonov, V.V., Jurka, J., Pačes, V.: HERVd: the Human Endogenous RetroViruses Database: update. *Nucleic Acids Res 32: D50, 2004.*

4. Zíka, R., Pačes, J., Pavlíček, A., Pačes, V.: WAViS server for handling, visualization and presentation of multiple alignments of nucleotide or amino acids sequences. *Nucleic Acids Res 32: W48-9, 2004.*

5. Pačes, J., Zíka, R., Pačes, V., Pavlíček, A., Clay, O., Bernardi, G.: Representing GC variation along eukaryotic chromosomes. *Gene 333: 135-141, 2004.*

6. Trtková, K., Plachý, J.: Deletions in the DNA binding domain of the *TP53* gene in v-*src*-transformed chicken cells. *In Vitro Cell Dev. Biol. Anim. 40: 285-292, 2004.*

7. Bobek, V., Plachý, J., Pinterová, D., Kolostová, K., Boubelík, M., Jiang, P., Yang, M., Hoffman, R.M.: Development of a green fluorescent protein metastatic-cancer chick-embryo drug-screen model. *Clin. Exp. Metastasis 21: 347-352, 2004.*

8. Bruhl, H., Cihak, J., Schneider, M.A., Plachy, J., Rupp, T., Wenzel, I., Shakarami, M., Milz, S., Ellwart, J.W., Stangassinger, M., Schlondorff, D., Mack, M.: Dual role of CCR2 during initiation and progression of collagen-induced arthritis: evidence for regulatory activity of CCR2+ T cells. *J. Immunol. 172: 890-898, 2004.*

9. Caldwell, S.J., Kierzek, A., Arakawa, H., Bezzubov, Y., Zaim, J., Fiedler, P., Kutter, S., Blagodatski, A., Kostavska, D., Koter, M., Plachý, J., Carnici, P., Hayashizaki, Y., Buerstedde, J.-M.: Full-length cDNAs from bursal lymphocytes to facilitate gene function analysis. *Genome Biol. 6, R6.1-R6.9, 2004.*

10. Pastoreková, S., Závada, J.: Carbonic anhydrase IX (CA IX) as a potential target for cancer therapy. *Cancer Ther. 2: 245-261, 2004.*

11. Závadová, Z., Závada, J.: Carbonic anhydrase IX (CA IX) mediates tumour cell interactions with microenvironment. *Oncol. Rep. 13: 977-982, 2005.*

12. Elleder, D., Stepanets, V., Melder, D.C., Šenigl, F., Geryk, J., Pajer, P., Plachý J., Hejnar J., Svoboda J., Federspiel, M.J.: The receptor for the subgroup C avian sarcoma and leukosis viruses, Tvc, is related to mammalian butyrophilins, members of the immunoglobulin superfamily. *J. Virol. 79: 10408-10419, 2005.*

13. Matoušková, M., Blažková, J., Pajer, P., Pavlíček, A., Hejnar, J.: CpG methylation suppresses transcriptional activity of human syncytin-1 in non-placental tissues. *Exp. Cell Res. 312: 1011-1020, 2006.*

14. Trefil, P., Mičáková, A., Mucksová, J., Hejnar, J., Brillard, J.-P., Bakst, M.R., Kalina, J., Poplštein, M.: Restoration of spermatogenesis and male fertility by transplantation of dispersed testicular cells in the chicken. *Biol. Reprod., in press.*

15. Pajer, P., Pečenka, V., Králová, J., Karafiát, V., Průková, D., Zemanová, Z., Kodet, R., Dvořák, M.: Identification of potential human oncogenes by mapping the common integration sites in avian nephroblastoma. *Cancer Res. 66: 78-86, 2006.*

Molecular Virology

Head: Michal Dvořák

Research Fellows: Marta Dvořáková, Vít Karafiát, Petr Kašpar, Jarmila Králová

Graduate Students: Vladimír Čermák, Zuzana Hořejší, Petr Pajer, Václava Škopová

Research Assistant: Vladimír Pečenka (part time)

Undergraduate Students: Jan Kosla (until September 2005), Michaela Starostová (from October 2005)

Technicians: Sonja Mandíková, Karolína Műllerová, Lenka Pichlíková

Introduction

The research efforts of the group focus on four main goals. First, it is the understanding of the molecular mechanisms regulating cell fate determination of multipotent haematopoietic and neural cells. Second, we are interested in the regulation of terminal differentiation of haematopoietic, neural and myogenic cells. Third, we study the molecular mechanisms involved in the malignant transformation of haematopoietic cells, melanocytes and of the nephrogenic blastema. Fourth, we study the molecular mechanisms of photoinduced apoptosis and search for compounds that would enable targeted drug delivery into tumour cells.

In studies on cell fate determination, differentiation and malignant transformation of haematopoietic cells and neural cells we use primarily avian cells and tissues and c-*myb* and v-*myb* genes and their specific mutants as tools to modulate developmental processes in these cells. In studies on the nephrogenic blastema transformation we use the model of the chicken nephroblastoma induced by the MAV retroviruses. Porphyrin derivatives with various peripheral substituents are used for targeted drug delivery and for induction of cell death in cancer cells and tissues.

Results

We found that the v-Myb protein affects commitment of multipotential myelo/erythroid, as well as bipotential myeloid progenitors and we have defined the crucial role of the v-Myb leucine zipper region in specification of the myeloid fate of multipotent cells (1). Further studies revealed that v-Myb in cooperation with the basic FGF instructs haematopoietic progenitors to develop into the erythroid lineage (2). We have recently uncovered a so far unknown regulatory step in the formation of avian neural crest. Graded intracellular concentrations of c-Myb protein significantly influenced the phenotype of the early neural tube cells. The higher c-Myb concentrations activated formation of neural crest through activation of the epithelio-mesenchymal transition in the chicken embryo neural tube (3). This work has been done in cooperation with Prof. M. Grim, Institute of Anatomy, Charles University, Prague. We have also uncovered the interaction of Myb proteins with MyoD, one of crucial myogenic factors. The interaction of c-Myb with MyoD appears to inhibit MyoD transcriptional activity and myotube formation in precursors that have not reached critical cell density (4). Our work on nephrogenic blastema transformation induced *in vivo* by the integration of the MAV2 ret-

rovirus led to the identification of the *twist* regulator as one of genes that are likely to take part in the formation of renal tumours (5). Further experiments have identified additional genes (e.g. *plag1* and *fox P1*) that are often mutated in chicken nephroblastoma. We have noticed that these genes are also deregulated in human renal tumours. Thus, identification of genes frequently mutated in the avian model system can lead to the identification of human oncogenes (6). One line of our research dealing with malignant transformation of haematopoietic cells has been focused on the development of porphyrin-based compounds that could mediate transport of antisense oligonucleotides into primary leukaemic cells (7). The development, application and mechanism of action of porphyrin-based photosensitizers that upon photoactivation induce cell death in cancer cells and tissues resulted in a discovery of novel bis-cyclodextrin porphyrin derivative that efficiently eliminates experimental tumours in mice (8).

Members of the laboratory contributed in cooperation to the work done by other groups (9-12).

Important results were also obtained by a member of the group during her Ph.D. studies abroad (13, 14).

The work of the group was supported by the grant No. AV0Z50520514 and grant No. A500520608 from the Academy of Sciences of the Czech Republic, by the Grant Agency of the Czech Republic (grants Nos. 304/03/0463, 203/02/0420, 301/04/1315 and 204/03/H066), and by the Grant Agency of the Academy of Sciences of the Czech Republic - research grants Nos. A5052309, AV0Z5052915-I044 and AV0Z5052915-I045.

Publications

1. Karafiát, V., Dvořáková, M., Pajer, P., Králová, J., Hořejší, Z., Čermák, V., Bartůněk, P., Zenke, M., Dvořák, M.: The leucine zipper region of Myb oncoprotein regulates commitment of hematopoietic progenitors. *Blood 98: 3668-3676, 2001*.

2. Bartůněk, P., Pajer, P., Karafiát, V., Blendinger, G., Dvořák, M., Zenke, M.: bFGF signaling and v-Myb cooperate in sustained growth of primitive erythroid progenitors. *Oncogene 21: 400-410, 2002*.

3. Karafiát, V., Dvořáková, M., Krejčí, E., Králová, J., Pajer, P., Šnajdr, P., Mandíková, S., Bartůněk, P., Grim, M., Dvořák, M.: Transcription factor c-Myb is involved in the regulation of the epithelial-mesenchymal transition in the avian neural crest. *Cell. Mol. Life Sci. 62: 2516-2525, 2005.*

4. Kašpar, P., Pajer, P., Sedlák, D., Tamaoki, T., Dvořák, M.: c-Myb inhibits myogenic differentiation through repression of MyoD. *Exp. Cell Res. 309: 419-428, 2005.*

5. Pajer, P., Pečenka, V., Karafiát, V., Králová, J., Hořejší, Z., Dvořák, M.: The twist gene is a common target of retroviral integration and transcriptional deregulation in experimental nephroblastoma. *Oncogene 22: 665-673, 2003*.

6. Pajer, P., Pečenka, V., Králová, J., Karafiát, V., Průková, D., Zemanová, Z., Kodet, R., Dvořák, M.: Identification of potential human oncogenes by mapping the common viral integration sites in avian nephroblastoma. *Cancer Res. 66: 78-86, 2006.*

7. Králová, J., Dvořák, M., Král, V.: Novel cationic transport agents for oligonucleotide delivery into primary leukemic cells. *J. Med. Chem. 46: 2049-2056, 2003*.

8. Králová, J., Synytsya, A., Poučková, P., Koc, M., Dvořák, M., Král, V.: Novel porphyrin conjugates with a potent photodynamic antitumor effect: differential efficacy of mono- and bis- -cyclodextrin derivatives *in vitro* and *in vivo. Photochem. Photobiol., in press*

9. Bříza, T., Kejík, Z., Vašek, P., Králová, J., Martásek, P., Císařová, I., Král, V.: Chromophoric binaphthyl derivatives. *Org. Lett. 7: 3661-3664, 2005.*

10. Souček, P., Anzenbacher, P., Skoumalová, I., Dvořák, M.: Expression of cytochrome P450 genes in CD34+ hematopoietic stem and progenitor cells. *Stem Cells 23: 1417-1422, 2005*.

11. Elleder, D., Stepanets, V., Melder, D.C., Šenigl, F., Geryk, J., Pajer, P., Plachý, J., Hejnar, J., Svoboda, J., Federspiel, M.J.: The receptor for the subgroup C avian sarcoma and leukosis viruses, Tvc, is related to mammalian butyrophilins, members of the immunoglobulin superfamily. *J. Virol. 79: 10408-10419, 2005.*

12. Nanka, O., Valášek, P., Dvořáková, M., Grim, M.: Experimental hypoxia and embryonic angiogenesis. *Dev. Dyn. 235: 723-733, 2006.*

13. Hořejší, Z., Falck, J., Bakkenist, C.J., Kastan, M.B., Lukas, J., Bártek, J.: Distinct functional domains of Nbs1 modulate the timing and magnitude of ATM activation after low doses of ionizing radiation. *Oncogene 23: 3122-3127, 2004.*

14. Bártková, J., Hořejší, Z., Koed, K., Kramer, A., Tort, F., Zieger, K., Guldberg, P., Sehested, M., Nesland, J.M., Lukas, C., Orntoft, T., Lukas, J., Bártek, J.: DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature 434: 864-870, 2005.*

Transcriptional Regulation

Head: Zbyněk Kozmik

Research Fellows: Jitka Láchová, Iryna Kozmiková (from May 2005), Jana Krešlová, Ondřej Machoň, Tomáš Vacík (from July 2004)

Graduate Students: Naoko Fujimura, Jana Růžičková

Undergraduate Students: Veronika Gabanová, Kristýna Jonášová

Technician: Veronika Nosková

Introduction

Our interest is focused on transcriptional regulation and its role in development and evolution. We are particularly interested in genes that play a fundamental role in eye and brain morphogenesis. Among those are genes encoding Pax transcription factors and components of the Wnt signalling pathway.

Results

Metazoan eye is a fascinating organ to be used for evolutionary studies. Early morphological studies have suggested that eye has evolved multiple times during the course of evolution. In contrast, more recent genetic data indicate a central role of Pax6 in eye development in most of the animals. In addition, other genes acting dowstream of Pax6 in the regulatory cascade (e.g. Six, Eya or Dach genes) are also highly evolutionarily conserved (1). However, most of our current knowledge is based on experimental work done in vertebrates and *Drosophila melanogaster*. To gain deeper insight into eye evolution, we use a cnidarian, jellyfish (*Tripedalia cystophora*) (2).

The central nervous system is the most complex tissue in the organism. In mammalian embryogenesis, the brain arises from immature neural progenitors that progressively differentiate into neurons, glia cells and oligodendrocytes. Several projects in our lab are aimed to understand genetic processes that control formation of the mouse brain. Using transgenic mice, we study morphogenetic regulators of the cell fate, cell cycle and migration (3, 4). We isolate and grow in culture dish neural stem cells and we study their properties such as differentiation plasticity, response to proliferative signals and survival upon transplantation (5).

The work of the group was supported by the Center of Integrated Genomics (project No. LN00A079), Center for applied Genomics (project No. 1M6837805002), and the Grant Agency of the Czech Republic (grant No. 204/04/1358).

Publications

1. Kozmik, Z.: Pax genes in eye development and evolution. *Curr. Opin. Genet. Dev. 15: 430-438, 2005.*

2. Piatigorsky J., Kozmik, Z.: Cubozoan jellyfish: an Evo/Devo model for eyes and other sensory systems. *Int. J. Dev. Biol. 48: 719-729, 2004.*

3. Diep, D.B., Hoen, N., Backman, M., Machoň, O., Krauss, S.: Characterisation of the Wnt antagonists and their response to conditionally activated Wnt signalling in the developing mouse forebrain. *Brain Res. Dev. Brain Res. 153: 261-270, 2004.*

4. Backman, M., Machoň, O., Mygland, L., van den Bout, C.J., Zhong, W., Taketo, M.M., Krauss, S.: Effects of canonical Wnt signalling on dorso-ventral specification of the mouse telencephalon. *Dev. Biol. 279: 155-168, 2005.*

5. Machoň, O., Backman, M., Krauss, S., Kozmik, Z.: The cellular fate of cortical progenitors is not maintained in neurosphere cultures. *Mol. Cell Neurosci. 30: 388-397, 2005.*

Cell Differentiation

Head: Petr Bartůněk

Research Fellow: Martina Zíková

Graduate Student: David Sedlák[1]

Research Assistant: Jana Bartůňková (part time)

Technician: Petra Szendzielarzová (from February 2005)

[1]Supported in part by Faculty of Science, Charles University, Prague

Introduction

Differentiation of haematopoietic stem cells and progenitors into various lineages is controlled by a complex array of extrinsic and intrinsic factors. Myeloid and erythroid cells develop from a common myeloid progenitor, which differentiates into either thrombocytes and erythrocytes, or granulocytes and macrophages. We are interested in three types of regulators of self-renewal and differentiation in hematopoietic cells, namely, nuclear hormone receptors, growth factors and their receptors, and cell cycle regulators. We have focused on the identification and analysis of thyroid hormone-regulated genes during thyroid hormone-accelerated erythropoiesis and their role in self-renewal and differentiation of erythroid cells. We also studied the prolyl-isomerase Pin1, an important mediator of phosphorylation-dependent signaling.

Finally, we identified and characterized the avian thrombopoietin and its receptor c-Mpl.

Our laboratory closely collaborated with the group of Michal Dvořák (Molecular Virology) and with Martin Zenke (RWTH, Aachen, Germany).

Results

Nuclear hormone receptors (NHR) comprise a large family of transcription factors that are involved in the regulation of diverse biological processes such as cell growth, differentiation, development and homeostasis. Recently we have established the model of thyroid hormone (T3)-accelerated erythroid differentiation to search for new thyroid hormone-regulated genes. The model is based on normal bone marrow erythroid progenitors expressing TRa/c-erbA at higher than endogenous levels. TRa/c-erbA in cooperation with SCF/c-kit signalling and in the absence of T3 facilitates sustained progenitor cell growth (self-renewal). Upon differentiation induction these cells have the capacity to develop into fully mature erythrocytes. This process is however dramatically accelerated by T3, implicating that T3 would induce or repress genes and respective pathways responsible for faster erythroid maturation and cell cycle withdrawal associated with terminal differentiation. We used the differential screening strategy to isolate genes directly regulated by T3. We have focused on one selected target, Trup1, a new member of the sterol-sensing domain protein family, to study its biological relevance. In addition to these studies utilizing the chicken model we have collaborated with the laboratory of Martin Zenke (RWTH, Aachen, Germany) to search for genes regulated by T3 during normal human erythroid differentiation employing Affymetrix DNA chips (Koh et al., submitted). We have concentrated on one selected target

– *GAR22* (Gas2-related gene on chr. 22) to study its role in cell growth arrest induced by T3.

Pin1 is a small peptidyl-prolyl cis/trans isomerase with the ability to bind and isomerase certain phosphorylated Ser/Thr-Pro bonds in the proteins; many of them are involved in the regulation of the cell cycle progression. We have established bicistronic retroviral-based expression system that allows us to identify new targets/substrates of Pin1 and thus shed more light on the role of Pin1 in cell proliferation and differentiation or leukaemia formation.

Recently we have identified, cloned and characterized the first non-mammalian thrombopoietin (Tpo) and its receptor c-Mpl (Bartunek et al., in preparation). The chicken Tpo expression is detectable in the liver and spleen and c-mpl is expressed mainly in the bone marrow and spleen. *In vitro* experiments with sorted multipotent chicken bone marrow-derived progenitors demonstrate the committing role of Tpo for the thrombocytic lineage and in cooperation with stem cell factor (SCF) also its role in self-renewal of multipotent progenitors. Using the *in vivo* approach, we demonstrated that intravenous application of recombinant chicken Tpo, or alternatively Tpo or c-mpl overexpresion via retroviruses lead to erythroblastosis and thromboblastosis in experimental animals. In addition to committed progenitors or development of the thrombocytic lineage, activation of the Tpo/c-Mpl pathway also affects very early multipotent haematopoietic progenitors.

The work of the group was supported by the VolkswagenStiftung (grant No. I/77 849), by the EMBO/HHMI Scientist Award to P. B., and in part by the Grant Agency of the Czech Republic (204/03/H066) (D.S.).

Publications

1. Bartůněk, P., Králová, J., Blendinger, G., Dvořák, M., Zenke, M.: GATA-1 and c-myb crosstalk during red blood cell differentiation through GATA-1 binding sites in the c-myb promoter. *Oncogene 22, 1927-1935, 2003.*

2. Macurek L., Ziková M., Dráberová E., Dráber P.: Monoclonal antibody BF-06 against the heavy chain of clathrin. *Folia Biol. (Praha) 49, 238-240, 2003.*

3. Zíková, M., DaPonte J.P., Dastugue, B., Jagla, K.: Patterning of the cardiac outflow region in Drosophila. *Proc. Natl. Acad. Sci. USA 100, 12189-12194, 2003.*

4. Kašpar, P., Pajer, P., Sedlák, D., Tamaoki, T., Dvořák, M.: c-Myb inhibits myogenic differentiation through repression of MyoD. *Exp. Cell Res. 309, 419-428, 2005.*

5. Karafiát, V., Dvořáková, M., Krejčí, E., Králová, J., Pajer, P., Šnajdr, P., Mandíková, S., Bartůněk, P., Grim, M., Dvořák, M.: Transcription factor c-Myb is involved in the regulation of the epithelial-mesenchymal transition in the avian neural crest. *Cell. Mol. Life Sci. 62, 2516-2525, 2005.*

Cell Signalling and Apoptosis

Head: Ladislav Anděra

Research Fellows: Vladimír Kořínek, Jan Kovář (part-time), Zuzana Naďová (until December 2005), Jiří Neužil (part-time)

Graduate Students: Lukáš Čermák, Jan Lukáš, Šárka Šímová, Tomáš Valenta, Martin Klíma, Lenka Doubravská, Michal Koc, Marie Ehrlichová, Karel Vališ, Jitka Neubaerová (until December 2005), Jan Švadlenka (from October 2005), Vladislava Šourková (from October 2005), Jaromíra Chladová (from October 2005)

Undergraduate Students: Bohumil Fafílek, Jitka Zájedová, Renata Neužilová, Vendula Pospíchalová

Technicians: Simona Benešová, Irena Lorintzová (until August 2005)

Introduction

Research in our laboratory is mainly focused on the elucidation of signal transduction from membrane receptors that affect cell growth, differentiation and/or apoptosis.

One of our major projects addresses the mechanism, regulation and possible practical exploration of signalling from the group of Death Receptors (DRs) of the Tumour Necrosis Factor Receptor (TNFR) family, namely from receptors for TRAIL (TNF Related Apoptosis Inducing Ligand) and from Death Receptor 6 (DR6). Intracellular parts of these receptors contain a protein-protein interaction region called the death domain and the major outcome of their signalling is induction of apoptosis. Apoptosis/caspase-independent signalling from these receptors can also lead to activation of SAP/MAP kinases and/or NF B. In addition to the death receptors, other membrane receptors such as leukosialin/CD43 can, upon their activation, signal apoptosis or affect apoptosis induced by TRAIL receptors. We currently investigate the potential role of Fas- and CD43-interacting adapter protein Daxx, the regulation of stress-induced apoptosis and transcription.

Another major signalling pathway studied in our group is the Wnt/Frizzled system. Secreted Wnt growth factors control numerous developmental processes and aberrant activation of Wnt signalling is also implicated in cellular transformation and cancer. The interaction of extracellular Wnt ligands with the Frizzled/LRP receptor complex results in increased intracellular levels of -catenin in the target cell. -catenin then translocates into the nucleus and in complex with TCF/LEF proteins it activates expression of specific Wnt responsive genes. We search for proteins that participate in this Wnt-TCF signalling axis and we analyse the interplay between the Wnt-induced and other cell signalling pathways.

Small molecules/therapeutic drugs can affect the outcome of DRs-induced apoptosis. We characterize the presumed interplay between vitamin E succinate (-TOS), recently emerged potential anti-tumour drug, and Death Ligands (FasL, TRAIL, TNF) in inducing apoptosis of tumour cells.

Iron is an essential component of the cell metabolism and is imported into cells via transferrin-dependent as well as independent pathways. Iron deprivation induces apoptosis of some tumour cells, while others are being resistant to it. Lack of iron also affects the cell cycle. We analyse the effect of iron-connected processes (transport, metabolism, iron deprivation) on proliferation and induction of apoptosis of various tumour cells.

Results

We have prepared several variants of the soluble cytotoxic ligand TRAIL and showed that it alone and in combination with a novel chemotherapeutical agent -tocopherol succinate (-TOS) efficiently induces apoptosis of a number of tumour-derived cell lines and primary leukaemia cells (1,2). Other drugs, chemicals such as ethanol or docosahexaenoic acid also cooperate with TRAIL in inducing apoptosis of tumour cells (3,4). In contrast, downregulation of oncogenic HER2 signalling suppresses TRAIL-induced apoptosis of breast cancer cells (5). Oncogenes, such as H-Ras, apparently in addition to their pro-proliferative activities can also sensitize tumour cells to death-inducing stimuli. We have demonstrated that H-Ras-transformed colon cancer cells upregulate expression of TRAIL receptors and are more sensitive to TRAIL-induced apoptosis than their non-transformed counterparts (6). Currently we investigate the mechanism of TRAIL-induced apoptosis and the effect of concurrent signalling (e.g. Wnt) on it. We also examine the mechanism and function of DR6-activated signalling and characterize apoptosis- and transcription-regulating activities of the adapter protein Daxx.

We have also demonstrated that iron deprivation leads to p53-independent apoptosis of some tumour cell lines (7) and that iron deprivation-induced apoptosis proceeds through the mitochondrial apoptotic pathway requiring translocation of Bax into mitochondria (8).

To study the regulatory mechanisms of Wnt signalling, we began a screen for proteins interacting with human TCF-4. We found that *C-terminal binding protein 1* (*CtBP1*), a widely expressed transcriptional co-repressor, interacts with the C-terminus of TCF-4 and suppresses Wnt-induced signalling in Hek/293 cells (9). At present, we analyse the role of other Tcf-4 interacting proteins such as tumour suppressor protein HIC 1 in Wnt-induced signalling and the importance of posttranslational modifications of Wnt ligands in initiating the signalling process.

The work of the group is supported by the Grant Agency of the Czech Republic (grants 204/04/0532, 305/04/0403), Grant Agency of the Academy of Sciences of the Czech Republic (grants A5052402, A5052304, B5052407, B552401), Ministry of Education, Youth and Sports of the Czech Republic (projects 1M6837805001 and EUREKA OE138) and Ministry of Health (projects NR8317-4 and NL7567-3).

Publications

1. Tomasetti, M,, Rippo, M.R., Alleva, R., Moretti, S., Anděra, L., Neužil, J., Procopio, A.: -Tocopheryl succinate and TRAIL selectively synergise in induction of apoptosis in human malignant mesothelioma cells. *Br. J. Cancer 90: 1644-1653, 2004.*

2 Stapelberg, M., Gellert, N., Swettenham, E., Tomasetti, M., Witting, P.K., Procopio, A., Neužil, J.: -Tocopheryl succinate inhibits malignant mesothelioma by disrupting the fibroblast growth factor autocrine loop: mechanism and the role of oxidative stress. *J. Biol. Chem. 280: 25369-25376, 2005.*

3. Vaculová, A., Hofmanová, J., Souček, K., Anděra, L., Kozubík, A.: Ethanol acts as a potent agent sensitizing colon cancer cells to the TRAIL-induced apoptosis. *FEBS Lett. 577: 309-313, 2004.*

4. Vaculová, A., Hofmanová, J., Anděra, L., Kozubík, A.: TRAIL and docosahexaenoic acid cooperate to induce HT-29 colon cancer cell death. *Cancer Lett. 229: 43-48, 2005.*

5. Dubská, L., Anděra, L., Sheard, M.A.: HER2 signaling downregulation by trastuzumab and suppression of the PI3K/Akt pathway: an unexpected effect on TRAIL-induced apoptosis. *FEBS Lett. 579: 4149-4458, 2005.*

6. Drosopoulos, K.G., Roberts, M.L., Čermák, L., Sasazuki, T., Shirasawa, S., Anděra, L., Pintzas, A.: Transformation by oncogenic RAS sensitizes human colon cells to TRAIL-induced apoptosis by up-regulating death receptor 4 and death receptor 5 through a MEK-dependent pathway. *J. Biol. Chem. 280: 22856-22867, 2005.*

7. Truksa, J., Kovář, J., Valenta, T., Ehrlichová, M., Polák, J., Naumann, P.W.: Iron deprivation induces apoptosis independently of p53 in human and murine tumour cells. *Cell Prolif. 36: 199-213, 2003.*

8. Koc, M., Naďová, Z., Truksa, J., Ehrlichová, M., Kovář, J.: Iron deprivation induces apoptosis via mitochondrial changes related to Bax translocation. *Apoptosis 10: 381-93, 2005.*

9. Valenta, T., Lukáš, J., Kořínek, V.: HMG box transcription factor TCF-4's interaction with CtBP1 controls the expression of the Wnt target Axin2/Conductin in human embryonic kidney cells. *Nucleic Acids Res. 31: 2369-2380, 2003.*

Signal Transduction

Head: Petr Dráber

Research Fellows: Michael Boubelík (part time), Lubica Dráberová, Ivana Hálová (part time)

Graduate Students: Viktor Bugajev, Petr Heneberg, Pavel Lebduška, Daniel Smrž, Magda Tůmová

Research Assistants: Romana Budovičová, Marek Dráber (part time),

Undergraduate Students: Peter Dráber (until July 2005)

Technicians: Dana Lorenčíková, Lukáš Kocanda, Hana Mrázová

Mast cells are specialized secretory cells that release inflammatory mediators in response to antigens, microorganisms, cytokines and other stimuli. Activation of mast cells significantly contributes to the development of inflammatory diseases such as asthma and related allergies. The key to any strategy to modulate inflammatory diseases is the understanding of the signal transduction pathways that control mast cell function. The work in this laboratory is focused on characterization of the biochemical processes that integrate signals from antigen receptors, cytokine receptors, and other surface receptors and regulate mast cell function. The laboratory has an integrated research programme using genetical, immunochemical and imaging approaches to explore mast cell signal transduction.

The most important result of the laboratory in the last two years was discovery of several new regulatory events involved in antigen-mediated activation of mast cells. We analysed functions of two palmitoylated molecules considered to be associated with lipid rafts, **Lyn** kinase and adaptor protein **NTAL** (non-T cell activation linker), the role of filamentous actin (**F-actin)** and the role of **topography of signalling molecules** on signal transduction.

Lyn kinase. Although it has been known for several years that the Src family tyrosine kinase Lyn initiates the Fc receptor I (Fc RI) signalling by phosphorylation of the receptor subunits, regulation of Lyn kinase activity and its consequences for the receptor signalling were not completely understood. To approach this problem, we prepared new phospho-specific antibodies to Lyn and showed, surprisingly, an increased phosphorylation of Lyn C-terminal regulatory tyrosine and decreased Lyn kinase activity during Fc RI-mediated mast cell activation. In accordance with these data we found that Lyn defective in the C-terminal tyrosine constitutively phosphorylated several substrates in resting cells, but did not cause Fc RI internalization or spontaneous degranulation. Fc RI-induced signalling in the presence of constitutively active Lyn resulted in an enhanced phosphorylation of the receptor subunits, Syk kinase, adaptor protein LAT (linker for activation of T cells), and production of phosphatidylinositol 1,4,5-trisphosphate (IP_3). In contrast, the subsequent activation of phospholipase C (PLC) 1, mobilization of intracellular calcium and degranulation were suppressed. Additionally, the constitutively active Lyn was strikingly less efficient than the wild-type Lyn in restoring the receptor-mediated calcium responses in bone marrow mast cells (BMMC) derived from $Lyn^{-/-}$ mice. These findings pinpoint the tight regulation of Lyn kinase activity as a critical event in mast cell degranulation.

NTAL. Antigen-initiated activation events include tyrosine phosphorylation of two transmembrane adaptor proteins, LAT and NTAL. Previous studies showed that the secretory response was partially inhibited in BMMC from LAT-deficient mice. To clarify the role of NTAL in mast cell degranulation, we compared Fc RI-mediated signalling events in BMMC from NTAL$^{-/-}$ and wild-type mice. Although NTAL is structurally similar to LAT, antigen-mediated degranulation responses were unexpectedly increased in NTAL-deficient mast cells. The earliest event affected was enhanced tyrosine phosphorylation of LAT in antigen-activated cells. This was accompanied by enhanced tyrosine phosphorylation and enzymatic activity of PLC 1 and PLC 2, resulting in elevated levels of IP_3 and free intracellular Ca^{2+}. NTAL$^{-/-}$ BMMC also exhibited an enhanced activity of phosphatidylinositol 3-OH kinase and SHP-2 phosphatase. Although both LAT and NTAL are considered to be localized in lipid rafts, immunogold electron microscopy on isolated membrane sheets demonstrated their independent clustering. The combined data show that NTAL is functionally and topographically different from LAT.

F-actin. Activation of mast cells is also dependent on the presence of F-actin. Previous studies using inhibitors of actin polymerization, cytochalasins and latrunculin B, revealed that F-actin has a negative regulatory role in Fc RI signalling. However, how F-actin is involved in regulating the activation remained enigmatic. Therefore, we have investigated the role of F-actin in mast cell triggering induced by aggregation of the glycosylphosphatidylinositol (GPI)-anchored proteins, Thy-1 and TEC-21, and compared it to activation via Fc RI. Pretreatment of rat basophilic leukaemia cells with latrunculin B inhibited the Thy-1-induced actin polymerization and elevated the Thy-1-mediated secretory and calcium responses. Inhibition of actin polymerization followed by Thy-1 aggregation resulted in increased tyrosine phosphorylation of Syk kinase, PLC , and LAT adapter, and some other signalling molecules. Enzymatic activities of phosphatidylinositol-3-kinase, PLC and phosphatase SHP-2 were also upregulated, but tyrosine phosphorylation of ezrin was inhibited. Similar changes were observed in Fc RI-activated cells. Significant changes in intracellular distribution, tyrosine phosphorylation and/or enzymatic activities of signalling molecules occurred in latrunculin-pretreated cells before cell triggering. Based on these data we have concluded that actin polymerization is critical for setting the thresholds for mast cell signalling via aggregation of both Fc RI and GPI-anchored proteins.

Topography of signalling molecules. Although aggregation of the Fc RI is necessary for antigen-mediated mast cell triggering, the relationship between the extent of the Fc RI aggregation and subsequent biochemical and topographical events is not completely understood. In further study we therefore analysed the activation events induced by Fc RI dimers, elicited by binding of anti-Fc RI monoclonal antibody to rat basophilic leukaemia cells. We found that in contrast to extensively aggregated Fc RI, receptor dimers (1) induced a less extensive association of Fc RI with detergent-resistant membranes, (2) delayed the tyrosine phosphorylation and membrane recruitment of several signalling molecules, (3) triggered a slower but more sustained increase in concentration of free cytoplasmic calcium, (4) induced degranulation, which was not inhibited at higher concentrations of the crosslinking antibody, and (5) failed to produce clusters of Fc RI, Syk kinase and Grb2 adapter in osmiophilic membranes as detected by immunogold electron microscopy on membrane sheets. Despite striking differences in the topography of Fc RI dimers and multimers, biochemical differences were less pronounced. The combined data suggest that Fc RI-activated mast cells propagate signals from small signalling domains formed around dimerized/oligomerized Fc RI,

and that the formation of large Fc RI aggregates in osmiophilic membranes promotes both strong receptor triggering and rapid termination of the signalling responses by receptor internalization.

We also wrote several reviews and commentary articles and participated in a collaborative study on the functional role of signalling molecules in mast cell activation.

Publications

1. Kyselová, V., Pěknicová, J., Boubelík, M., Buckiová, D.: Body and organ weight, sperm acrosomal status and reproduction after genistein and diethylstilbestrol treatment of CD1 mice in a multigenerational study. *Theriogenol. 61: 1307-1325, 2004.*

2. Lusková, P., Dráber, P.: Modulation of the Fc receptor I signaling by tyrosine kinase inhibitors: search for therapeutic targets of inflammatory and allergy diseases. *Curr. Pharm. Des. 10: 1727-1737, 2004.*

3. Volná, P., Lebduška, P., Dráberová, L., Šímová, Š., Heneberg, P., Boubelík, M., Bugajev, V., Malissen, B., Wilson, B.S., Hořejší, V., Malissen, M., Dráber, P.: Negative regulation of mast cell signaling and function by the adaptor LAB/NTAL. *J. Exp. Med. 200: 1001-1013, 2004.*

4. Tkaczyk, C., Hořejší, V., Shoko, I., Dráber, P., Samelson, L.E., Satterthwaite, A.B., Nahm, D.H., Metcalfe, D.D., Gilfillan, A.M.: NTAL phosphorylation is a pivotal link between the signaling cascades leading to human mast cell degranulation following kit activation and Fc RI aggregation. *Blood 104: 207-214, 2004.*

5. Dráberová, L., Lebduška, P., Hálová, I., Tolar, P., Štokrová, J., Tolarová, H., Korb, J., Dráber, P.: Signaling assemblies formed in mast cells activated via Fc receptor I dimers. *Eur. J. Immunol. 34: 2209-2219, 2004.*

6. Tolarová, H., Dráberová, L., Heneberg, P., Dráber, P.: Involvement of filamentous actin in setting the threshold for degranulation in mast cells. *Eur. J. Immunol. 34: 1627-1636. 2004.*

7. Tolar, P., Dráberová, L., Tolarová, H., Dráber, P.: Positive and negative regulation of Fc receptor I-mediated signaling events by Lyn kinase C-terminal tyrosine phosphorylation. *Eur. J. Immunol. 34: 1136-1145, 2004.*

9. Heneberg, P., Dráber, P.: Regulation of Cys-based protein tyrosine phosphatases via reactive oxygen and nitrogen species in mast cells and basophils. *Curr. Med. Chem. 12: 1859-1871, 2005.*

10. Dráber, P., Dráberová, L.: Lifting the fog in store-operated Ca^{2+} entry. *Trends Immunol. 26: 621-624, 2005.*

11. Bobek, V., Boubelík, M., Fišerová, A., Luptovcova, M., Vannucci, L., Kacprzak, G., Kolodzej, J., Majewski, A.M., Hoffman, R.M.: Anticoagulant drugs increase natural killer cell activity in lung cancer. *Lung Cancer 47: 215-223, 2005.*

12. Bobek, V., Pintérová, D., Kološtová, K., Boubelík, M., Douglas, J., Teyssler, P., Pavlásek, J., Kovařík, J.: Streptokinase increases the sensitivity of colon cancer cells to chemotherapy by gemcitabine and cis-platine in vitro. *Cancer Lett., in press.*

Intracellular Communications

Head: Zdena Tuháčková

Research Fellows: Dana Kučerová, Vlasta Sovová (until December 2004), Eva Šloncová, Jolana Turečková, Martina Vojtěchová

Undergraduate Students: Alexandra Bukovská (until July 2005), Helena Handrková

Technicians: Miloslava Čechová, Iva Dosoudilová

Introduction

The fundamental principle of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells. Several signal transduction pathways are deregulated in human cancers. This is associated with changes in the activity of signalling proteins including transducers and activators of protein kinase cascades. To characterize the processes that may be responsible for malignant transformation of cells, we have studied several molecular mechanisms that control intracellular signalling in tumour and normal cells. In particular, we analysed human colon cancer cells **(A)** and model hamster cells transformed by the v-*src* oncogene **(B)**.

(A) The availability of several cell lines derived from human colorectal carcinomas and the possibility to induce their transient differentiation in response to sodium butyrate or glucose deprivation, when cancer cells exhibit many biochemical and morphological features of normal enterocytes, offer a unique opportunity to analyse the role of several molecular effectors in mitogenic and oncogenic potential of human tumour cells. One of these effector proteins, -catenin, can alter transcription of several genes responsible for cell proliferation, besides its role in cadherin-mediated cell adhesion, and may play an important role in carcinogenesis of colonocytes. -catenin is a substrate for several protein kinases and its phosphorylation that is catalyzed by glycogen synthase kinase-3 (GSK-3), one of the key regulatory proteins in the phosphoinositide-3 kinase (PI-3K)-dependent signalling pathway, results in -catenin degradation. Also E-cadherins, in addition to their contribution to cell adhesion, may play a role in signal transduction. It has been shown recently that in some type of cells, E-cadherin can induce the ligand-independent activation of EGF receptor (EGFR), probably by means of their co-clustering. The interactions and activation of these proteins were therefore analysed in colorectal cell lines. The regulatory role of PKC II in proliferation and differentiation of colon cancer cells was also examined.

(B) Perturbation of Src signalling results in alterations of Src activity, deregulation of cell growth and malignant transformation. Increased protein synthesis is necessary for cancer progression to support a rapid proliferation of cancer cells. Translational control of mRNA expression is a major regulatory mechanism of protein synthesis, which is mediated by the interactions between molecules involved in cellular signalling and components of the translational apparatus. Translation has an established role in cell growth. Until recently, however, little was known about the alterations in mRNA translation in cancer and about their role in the development and progression of cancer. One of the signal transduction pathways that led to translational control of gene expression is a signalling pathway in which the central regulatory protein is mTOR (mammalian target of rapamycin). A macrolide antibiotic, rapamycin, specifically inhibits

mTOR, resulting in reduced cell growth, a reduced rate of cell cycle progression and a reduced rate of proliferation. The mTOR-dependent signalling pathway was found to be activated during malignant transformation and cancer progression in many human cancers. This pathway selectively controls translation of several genes related to growth and cell progression. Their mRNAs are poorly translated in quiescent cells but preferably recruited to ribosomes after a mitogenic signal. The main factors contributing to low efficiency of mRNA translation include structural elements within particular mRNAs, such as the presence of a polypyrimidine-rich motif (5'TOP) at the 5'terminus of mRNA and a high 5'UTR secondary structure of mRNA, mainly implicated in the regulation of cap-dependent translation. Rapamycin selectively inhibits initiation of translation, probably by blocking the function of the cap-binding protein eIF4E, which is regulated by the translation repressor proteins (4E-BP1,2), and the activity of ribosomal S6 protein kinase p70 S6K that is involved in the recruitment of 5'TOP mRNAs to actively translating polysomes upon mitogenic stimulation. The proteins encoded by 5'TOP genes have been shown to act themselves as protooncogenes. Their enhanced expression may induce malignant transformation or alter the cell growth. Therefore, deregulation in 5'TOP gene expression may have direct consequences on cancer initiation.

Another signalling pathway that is activated by growth-promoting stimuli is a rapamycin-insensitive pathway, which is implicated in both cell survival and death by activating multiple distinct signalling cascades downstream to phosphoinositide-3 kinase (PI-3K).

Tyrosine kinase activity of Src is elevated in several types of human cancer, which has been attributed to both enhanced Src expression and increased specific activity. To explore the mechanisms that are involved in the process of cell malignancy, we have analysed hamster fibroblasts that were transformed by Rous sarcoma virus (RSV). Cellular responses to a highly activated Src protein, which is overexpressed in RSV-transformed cells, may help recognize the mechanisms by which cells are transformed to the malignant phenotype.

Results

(A) Biochemical and ultrastructual analyses of induced differentiation in HT29 cells showed that their response to sodium butyrate (NaBT) depends on the time of exposure and glucose deprivation. A more differentiated phenotype with changes of the subcellular structures leading to apoptosis was found upon treatment with NaBT and glucose deprivation. The different types of mitochondria that were seen in HT29 cells after their treatment with differentiation-inducing agents suggest a pleiotropic response of this cancer cell line, obviously depending on the differentiated state of the cells and particular culture conditions (1).

The frequency and intensity of the expression of several molecular markers of cell malignant transformation was examined in colorectal cancer and neighbouring mucosa. They reflected the complex character of carcinomatous transformation of the epithelial cells (2).

Upon EGF treatment, we have observed activation of the PI-3K/Akt/GSK3 signalling pathway in several colorectal cancer cells, indicating that EGFRs are functional even in the cells containing a low density of EGFRs. However, the transactivation of EGFR by E-cadherin did not seem to occur in the analysed cells independently of the amount of EGFR molecules present in these cells (3).

The activity of PI-3K and PKC II decreased in HT29 cells differentiating upon treatment with sodium butyrate. Ectopic constitutively active PI-3K potentiated the activity of PKC II and blocked differentiation measured by the activity of alkaline phosphatase. Surprisingly, it did not increase the activity of Akt – so far known as an obligatory downstream target of PI-3K. In Caco-2

cells differentiating spontaneously, PI-3K had no effect on the activity of alkaline phosphatase or PKC II. According to these results, different mechanisms of differentiation may exist in colorectal adenocarcinoma cells (4).

We have found that -catenin expression was downregulated upon inhibition of PI-3K activity by its selective inhibitor, wortmannin. Sodium butyrate overcame this effect at the translational level of -catenin expression and significantly increased phosphorylation of ribosomal protein S6, accompanied with the increased phosphorylation of p70 S6K and MAPKs, the effector proteins that are upstream of protein S6 in the distinct signalling pathways. These facts indicate that different signalling pathways may be involved in the regulation of -catenin synthesis. Moreover, the fact that modulation of -catenin expression by NaBT occurred at the level of protein translation suggests that sodium butyrate may act as a translational regulator (5). Since we had previously obtained variable results during analyses of HT29 cells, we established several clones of the HT29 cell line. Four of such clones were characterized (Figs. 1, 2). They differed in their response to NaBT regarding some markers of differentiation including morphological features evaluated by electron microscopy, expression of CEACAM-1 and activity of alkaline phosphatase or caspase-3. Our data showed considerable heterogeneity of the clones. This fact may contribute to the evidence that HT29 cells possess multipotent information similar to that of stem cells of the normal intestinal crypt (6).

(B) We have previously shown that the enhanced expression and activity of the Src protein correlated with increased levels of overall protein synthesis in RSV-transformed hamster fibroblasts, indicating that cellular transformation by the v-*src* oncogene leads to upregulation of protein synthesis. This is accompanied with the activation of at least two distinct signalling pathways, the mTOR signalling pathway and the PI-3K-dependent signalling pathway. Moreover, important distinctions exist between the signalling pathways that modulate protein synthesis at the level of translational control in non-transformed cells compared to RSV-transformed cells. A high basal activity of p70 S6K and the increased phosphorylation of its physiological substrate,

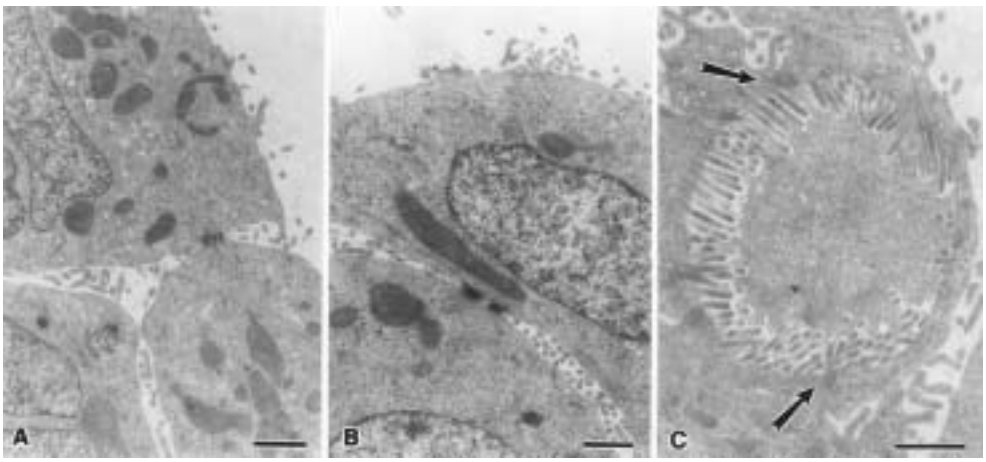


Fig. 1. Utrastructure of the cells of ***H8*** and ***G9*** clones. Undifferentiated features of ***H8*** and ***G9*** cells (*A* and *B*). Intercellular cyst in some cells of the ***H8*** clone (*C*). Tight junctional complexes are indicated by arrows. Bars = 1 m.

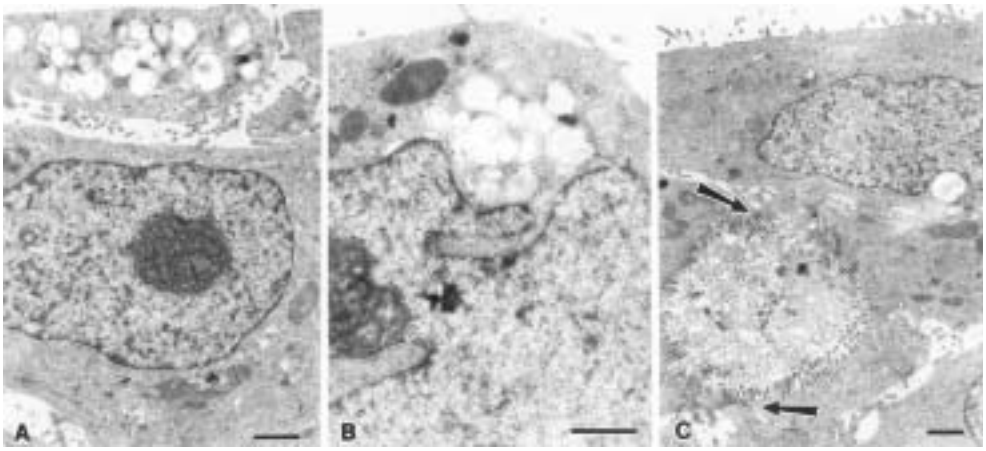


Fig. 2. Utrastructure of the cells of ***G10*** and ***A3*** clones. Goblet-like cells of ***G10*** and ***A3*** clones (*A* and *B*). Intercellular cyst in some cells of the ***G10*** clone (*C*). Tight junctional complexes are indicated by arrows. Bars = 1 m.

ribosomal protein S6, appear to be independent of PI-3K in RSV-transformed hamster fibroblasts, but are PI-3K-dependent in the normal hamster fibroblasts. In contrast, phosphorylation of the S6 protein and the activity of p70 S6K are controlled by the mTOR signalling pathway in both non-transformed and v-*src*-transformed cells. A distinct, rapamycin-insensitive signalling pathway of phosphoinositol 3-kinase (PI-3K), is also activated by transformation of the cells by the v-*src* oncogene. This pathway, however, appeared to be independent of the mTOR signalling pathway in v-*src*-transformed cells, while it is connected to mTOR in the normal non-transformed cells (7).

Our studies thus revealed two important differences between v-*src*-transformed and non-transformed cells in respect of the regulation of proteins that modulate selective and global protein synthesis and the signal transduction pathways controlling them. Since mTOR is emerging as a selective target for cancer therapy, inhibitors of Src activity may also display therapeutical efficiency against a variety of human tumours in which the mTOR signalling pathway is activated (8).

In vitro kinetic studies of the activity of v-Src and c-Src proteins, which are present in RSV-transformed fibroblasts, showed that the peptide substrate specificities of these two tyrosine kinases are different. This finding may reflect different protein substrate specificities of the v-Src and c-Src kinases *in vivo* that may be responsible for important differences between v-*src*-transformed and non-transformed cells (9).

Phosphorylation of the S6 protein of 40S ribosomal subunit, which is responsible for binding and decoding mRNA, is a common effect of mitogens regulating selective translation of 5'TOP mRNAs. In agreement with this, we demonstrated that growth-stimulation of the cytolytic T cells (CTLL2) with interleukin 2 (IL2) increased the phosphorylation of ribosomal protein S6, which was accompanied with the increased phosphorylation of the ribosomal S6 protein kinase p70 S6K. IL2-dependent activation of p70 S6K and phosphorylation of the S6 protein were completely blocked by rapamycin and significantly decreased upon pharmacological inhibition of PI-3K. Thus, unlike in v-*src*-transformed cells, the PI-3K signalling pathway in concert with the signalling pathway of mTOR is evident-

ly involved in IL2-dependent phosphorylation of the ribosomal protein S6. These data confirmed that S6 could be an important regulator of cell growth (10).

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 301/00/0269, 301/04/0550 and 301/02/D159) and the Grant Agency of the Academy of Sciences of the Czech Republic (research grant No. KJB5052302).

Publications

1. Sovová, V., Štokrová, J., Šloncová, E., Kučerová, D., Tuháčková, Z., Korb, J.: Alterations in mitochondria function and morphology in HT29 cells upon conditions inducing differentiation and apoptosis. *Int. J. Oncol. 23: 1755-1760, 2003*.
2. Frič, P., Sovová, V., Roth, Z., Šloncová, E., Kocna, P., Jirásek, A., Čermák, J.: Distribution of molecular markers in sporadic colorectal cancer, adjacent and distal mucosa. *Folia Gastroenterol. Hepatol. 2: 62-71, 2004*.
3. Sovová, V., Kučerová, D., Vojtěchová, M., Šloncová, E., Tuháčková, Z.: Transactivation of E-cadherin is not involved in the activity of EGF receptor in colorectal carcinoma cells. *Int. J. Oncol. 25: 1459-1464, 2004.*
4. Turečková, J., Vojtěchová, M., Kučerová, D., Velek, J., Tuháčková, Z.: Sodium butyrate-mediated differentiation of colorectal cancer cells: regulation of PKC betaII by PI 3-kinase. *Int. J. Mol. Med. 15: 329-35, 2005.*
5. Turečková, J., Kučerová, D., Vojtěchová, M., Šloncová, E., Tuháčková, Z.: Expression of -catenin is regulated by PI-3 kinase and sodium butyrate in colorectal cancer cells. *Int. J. Mol. Med. 17: 69-75, 2006.*
6. Štokrová, J., Šloncová, E., Sovová, V., Kučerová, D., Žíla, V., Turečková, J., Vojtěchová, M., Korb, J., Tuháčková, Z.: Characterization of four clones derived from human adenocarcinoma cell line HT29 and analysis of their response to sodium butyrate. *Int. J. Oncol. 28: 559-565, 2006.*
7. Vojtěchová, M., Šloncová, E., Kučerová, D., Jiřička, J., Sovová, V., Tuháčková, Z.: Initiation factor eIF2B not p70 S6 kinase is involved in the activation of the PI-3K signalling pathway induced by the v-*src* oncogene. *FEBS Lett. 543: 81-86, 2003.*
8. Tuháčková, Z.: Molecular therapeutics – lessons from the role of Src in cellular signalling. *Folia Biol. (Praha) 51: 114-120, 2005*.
9. Vojtěchová, M., Tuháčková, Z., Hlaváček, J., Velek, J., Sovová, V.: The v-Src and c-Src tyrosine kinases immunoprecipitated from Rous sarcoma virus-transformed cells display different peptide substrate specificities. *Arch. Biochem. Biophys. 421: 277-282, 2004.*
10. Tuháčková, Z., Šloncová, E., Vojtěchová, M., Sovová, V.: IL2-dependent phosphorylation of 40S ribosomal protein S6 is controlled by PI-3K/mTOR signalling in CTLL2 cells. *Int. J. Mol. Med. 13: 601-605, 2004.*

Biology of Cytoskeleton

Head: Pavel Dráber

Research Fellows: Eduarda Dráberová, Vladimír Viklický (Deputy Director of the Institute, part time), Vladimír Zelený (part time)

Graduate Students: Lenka Libusová (until December 2004), Libor Macůrek, Vadym Sulimenko, Barbora Šmejkalová

Research Assistants: Věra Richterová, Tetyana Sulimenko, Vladislava Marková (from August 2005)

Undergraduate Student: Jan Bražina

Technicians: Ivana Macháčková (part time), Lenka Josková (until April 2005)

Introduction

The long-term research programme of the laboratory has been focused on studying the structure-function relationships of microtubule proteins and their interactions with other cytoskeletal elements in cells under normal and pathological conditions. In cells, microtubules have essential functions that are carried out by populations of microtubules of different composition, stability and properties. Microtubules consist of evolutionarily conserved -tubulin heterodimes and a collection of microtubule-associated proteins (MAPs). Both tubulin subunits are encoded by multiple tubulin genes and tubulin post-translational modifications modulate interactions of microtubules with MAPs. Various signalling molecules interact with microtubules and microtubules are likely to be critical for the spatial organization of signal transduction. The organization of microtubular networks is controlled by microtubule organizing centres (MTOCs). One of the key components of MTOCs is -tubulin, which is necessary for nucleation and organization of microtubules. -Tubulin also exists in cells in the form of soluble -tubulin complexes and it is associated with membranous components. There are cumulative data indicating that -tubulin could have other functions than nucleation of microtubules. We have focused our efforts mainly on the elucidation of the molecular mechanisms governing the microtubule nucleation and the role of -tubulin in this process.

Results

Non-receptor protein tyrosine kinases of the Src family have been shown to play an important role in signal transduction as well as in regulation of microtubule protein interactions. We have shown that -tubulin in differentiating embryonic carcinoma cells is phosphorylated and forms complexes with protein tyrosine kinases of the Src family, Src and Fyn. *In vitro* kinase assays showed tyrosine phosphorylation of proteins in -tubulin complexes isolated from differentiated cells. Pretreatment of cells with a Src family selective tyrosine kinase inhibitor reduced the amount of phosphorylated -tubulin in the complexes. Binding experiments with recombinant SH2 and SH3 domains of Src and Fyn kinases revealed that protein complexes containing -tubulin bound to SH2 domains and that these interactions were of SH2-phosphotyrosine type. We suggest that Src family kinases might play an important role in the regulation of -tubulin interaction with tubulin dimers or other proteins (3).

Centrosome amplification is a pivotal mechanism underlying tumorigenesis. We have found that overexpression and ectopic cellular distribution of -tubulin is characteristic for astrocytic gliomas and glioblastoma cell lines. The ectopic distribution was different from pericentrin, centrosome-associated protein. Our results indicate that ectopic cellular expression of -tubulin in diffuse astrocytic gliomas may be significant in the context of centrosome dysfunction/amplification and may be linked to tumour progression, where it may potentially serve as a novel marker of anaplastic changes (7).

The mechanisms of microtubule nucleation and formation of microtubule arrays are conveniently studied on flagellated and ciliated protozoa models, as they contain multiple microtubular structures in single cells. We have shown that -tubulin has a unique and complex localization pattern during the cell cycle of flagellated *Leishmania* promastigotes. The cell cycle-dependent distribution of -tubulin in the posterior end of the cell could imply its important role in microtubule anchorage during interphase and mitosis. -Tubulin was found only in insoluble cytoskeletal fractions, in contrast to tubulin dimers, and multiple charge variants of -tubulin were discriminated. Posttranslational modifications of -tubulin are therefore phylogenetically conserved (1). In ciliated *Tetrahymena* and *Paramecium*, we have found discrete localization of a -tubulin epitope (81–95) in the cortical region outside the basal bodies. This is the first demonstration of subcellular sequestration of a -tubulin epitope in ciliates that does not reflect the known posttranslational modifications of tubulin (5). Current knowledge concerning the diversity of microtubular structures, tubulin genes, and posttranslational modifications in ciliates has been reviewed (6).

The specificity of new monoclonal antibodies against kinesin was determined using human recombinant kinesin heavy chain and its structural domains (4). We have also developed a fast and simple dot-immunobinding assay for quantification of a large number of samples of mouse immunoglobulins in hybridoma culture supernatants (2).

The work of the group was supported by the Grant Agency of the Czech Republic (Research Grants Nos. 204/05/2375 and 304/04/12733), the Grant Agency of the Academy of Sciences of the Czech Republic (Research Grant No. IBS5052301), by grants from the Ministry of Education (1PO4OE158 NEURO), EU (INTAS) project (No. 03-55-6459) and by projects LN00A026 (Center of Molecular and Cellular Immunology) and LC545 (Center of Cell Functional Organization) from the Ministry of Education, Youth and Sports of the Czech Republic.

Publications

1. Libusová, L., Sulimenko, T., Sulimenko, V., Hozák, P., Dráber, P.: Gamma-tubulin in Leishmania: cell-cycle dependent changes in subcellular localization and heterogeneity of its isoforms. *Exp. Cell Res. 295: 375-386, 2004.*

2. Sulimenko, T., Dráber, P.: A fast and simple dot-immunobinding assay for quantification of mouse immunoglobulins in hybridoma culture supernatants. *J. Immunol. Methods 289: 89-95, 2004.*

3. Kukharskyy, V., Sulimenko, V., Macůrek, L., Sulimenko, T., Dráberová, E., Dráber, P.: Complexes of -tubulin with non-receptor protein tyrosin kinases Src and Fyn in differentiating P19 embryonal carcinoma cells. *Exp. Cell Res. 298: 218-228, 2004.*

4. Malcová-Janatová, I., Richterová, V., Dráber, P., Hašek, J.: Preparation of human recombinant kinesin heavy chain and epitope mapping of its structural domains. *Folia Microbiol. (Praha) 49: 665-670, 2004.*

5. Libusová, L., Sulimenko, T., Sulimenko, V., Janisch, R., Hozák, P., Dráber, P.: Distinct localization of beta-tubulin epitope in *Tetrahymena thermophila* and *Paramecium caudatum* cortex. *Protoplasma 225: 157-167, 2005.*

6. Libusová, L., Dráber, P.: Multiple tubulin forms in ciliates. Review. *Protoplasma, in press.*

7. Katsetos, C.D., Reddy, G., Dráberová, E., Šmejkalová, B., Del Valle, L., Ashraf, Q., Tadevosyan, A., Yelin, K., Maraziotis, T., Mishra, O.P., Mörk, S., Legido, A., Nissanov, J., Baas, P.W., de Chadarévian, J.P., Dráber, P.: Altered cellular distribution and subcellular sorting of -tubulin in diffuse astrocytic gliomas and human glioblastoma cell lines suggest centrosome protein amplification. *J. Neuropathol. Exp. Neurol., in press.*

Cell Biology

Head: Eva Matoušková

Research Fellows: Pavel Veselý (part time), Eva Pokorná (on maternity leave)

Graduate Students: Luboslava Krásná (on maternity leave), Markéta Brožová (Ph.D. student), Eva Buršíková (Ph.D. student), Kristina Procházková (part time)

Research Assistants: Alena Chaloupková (part time)

Technicians: Eva Krchňáková (part time), Anna Bláhová (part time)

Introduction

The long-term research orientation of the laboratory is focused on *ex vivo* isolation of human epithelial primary cells derived from normal and tumour tissue, their propagation and characterization with attention to recognition of stem cells. The experimental work has been carried out in the following areas:

1) recombined human/pig skin: preparation and use in the clinical practice for treatment of burns and other skin defects

2) human mammary epithelial cells: normal and tumour primary cell isolation, cultivation and characterization in relation to the neoplastic disease

3) differentiation of mammary tumour progenitor cells

4) chemosensitivity/chemoresistance: evaluation of individual breast tumours

5) analysis of podosomal structures associated with neoplastic cells

Results

Deep dermal burns frequently tend to convert into full-thickness skin loss. We found that this wound deepening may be prevented by recombined human/pig skin (RHPS), consisting of human allogeneic keratinocytes cultured on cell-free pig dermis. RHPS, which shows a skin-like consistency, has to be applied on the burn wound „upside-down“, i.e. with the keratinocyte layer facing the wound. The wound has to be prepared by dermabrasion to the capillary bleeding level. In our practice, more than 70% of deeply dermabraded wounds covered with the recombined skin healed without scarring (Fig.1).

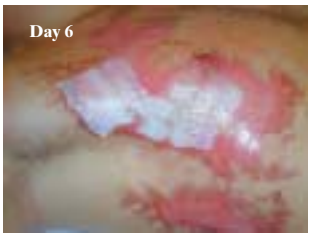
Breast cancer belongs among poorly understood human cancers. One of the reasons is lack of a culture system for maintaining malignant cells from primary breast tumours and solid metastases in *in vitro* conditions. We have achieved regular expansion of heterogeneous populations of luminal and myoepithelial cells from small biopsies of primary human breast tumours. From one of these populations a new clonal cell line, EM-G3, was derived. Fluorescence *in situ* hybridization (FISH) and multicolour FISH analysis demonstrated a stable diploid genome with minor genetic changes. The EM-G3 cells formed limited tumours in nu/nu mice organized in primitive ductal-like structures composed of luminal-like and myoepithelial-like cells. Our results indicate that EM-G3 cells were derived from a population of common progenitors of luminal and myoepithelial cells that were immortalized in an early stage of tumorigenesis (1 and submitted manuscript). *In vitro* drug resistance of cells cul-

Prevention of burn autotransplantation with recombined human/pig skin (RHPS)

A 18-year-old male patient was seriously burned with hot oil. On day 4 the wound deepened.

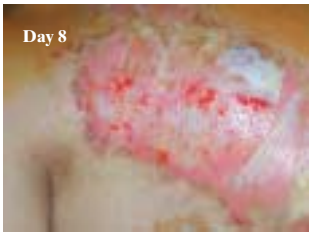
On day six the wound deepened to 2b–3 dg. The white area is necrosis, which usually needs autotransplantation

The wound treated with antibacterial creams was dermabraded with longitudinally applied forceps

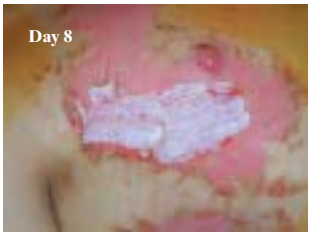


After reaching the level of capillary bleeding the wound was covered with RHPS (keratinocytes down)

Two days later the applied RHPS appeared as a dry brownish membrane



The dry RHPS was removed

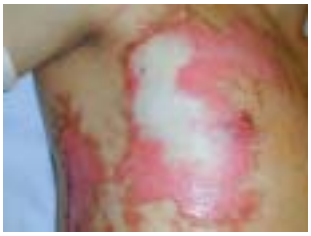


The deep part of the wound was covered with fresh PHPS (2nd application)

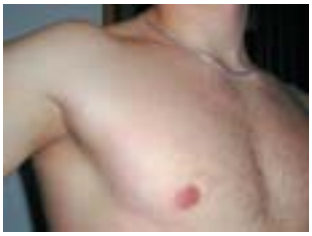
Two days after the 2nd application of RHPS the dry RHPS from the central area (the deepest part) was removed

The deepest part of the wound was again covered with fresh RHPS (3rd application)

Six days after the 3rd application of RHPS the wound was healed



The state of the wound before RHPS treatment



Final result

<u>Conclusion:</u> No operation, autotransplantation and anaesthesis was needed. The wound healed without any scar.

tured from mammary carcinomas has been tested by the MTT assay.

Podosomal structures in RsK4 tumour cells were studied *in vitro*. The results showed that different types of podosomal structures were associated with the cell shape and depended upon the environmental conditions. A possible route of involvement of podosomal structures in the uptake of nutrients by the cell was indicated. A phenotypical plasticity of the RsK4 neoplastic cells, which can also be functional and related to malignancy, was unveiled (2).

The group's work was supported by „The program for the support of the targeted research and development", Academy of Sciences of the Czech Republic (Project No. S5052312), by the Grant Agency of the Ministry of Health of the Czech Republic (Grants Nos. NR/8323-3, NR/8345-4 and NR/8145-3), by the Grant Agency of the Czech Republic (Grant No. 524/04/0102) and by the Internal Grant of Charles University for Ph.D. students (93/2005).

Publications

1. Matoušková, E., Kudláčková, I., Chaloupková, A., Brožová, M., Netíková, I., Veselý, P.: Origin of cells cultured in vitro from human breast carcinomas traced by cyclin D1 and HER2/neu FISH signal numbers. *Anticancer Res. 25: 1051-1058, 2005.*

2. Veselý, P., Blase, Ch., Matoušková, E., Bereiter-Hahn, J.: Arising podosomal structures are associated with neoplastic cell morphological phenotype induced by the microenvironment. *Anticancer Res., in press.*

Gene Expression

Head: Jiří Jonák

Research Fellows: Vladimír Fučík, Libor Krásný

Graduate Students: Hana Šanderová, Zoltán Ferjentsik, Radek Šindelka, Luděk Sojka (from October 2004)

Research Assistants: Elena Muchová (until June 2005)

Undergraduate Students: Luděk Sojka (until October 2004), Hana Tišerová, Martina Svobodová (from April 2004), Tomáš Kouba (from April 2005)

Technicians: Libuše Výborná, Eva Nosková, Jitka Dvořáková

Introduction

In the last two years the research in the laboratory was focused on three main topics:

(i) Gene expression in vertebrates during early stages of development
(ii) Gene expression in bacteria
(iii) Structure-function relationships in proteins

Ad (i) **Gene expression in vertebrates during early stages of development**

Model system: *Xenopus laevis*. Expression and role of its maternal and zygotic genes and genes of the Src family of tyrosine kinases.

The correct spatial and temporal expression of both maternal and zygotic mRNAs is necessary for the first developmental processes such as the main body axis formation, gastrulation, germ layer induction, etc. to proceed. Orchestrated changes in their levels are a key mechanism in cell and tissue differentiation. To be able to quantify the levels of individual mRNAs to reveal their mutual interplay and significance during development, the normalization with respect to the expression of various reference genes has been routinely carried out.

Until now, *Xenopus laevis* expression studies have most frequently used the genes coding for elongation factor eEF-1 , glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ornithine decarboxylase (ODC), L8-ribosomal protein and H4-histone as reference genes. We have determined developmental expression profiles of these genes by real-time RT-PCR. The experiments revealed great fluctuations in the expression of all these genes in the course of *X. laevis* early development (stages 0-44), and individual reference genes displayed different profiles. This indicates that developmental changes significantly compromise the relative mRNA quantification based on these reference genes when different developmental stages are to be compared. Instead, we have shown that for the quantification of mRNAs it is favourable and reliable to carry out normalization against total RNA, because its concentration in *X. laevis* embryo is subject to only negligible fluctuations and remains essentially stable throughout all early developmental stages, with only a slow increase after stage 32 (6).

These results made it possible to determine temporal developmental expression profiles of several *X. laevis* developmental genes. In total, 16 important genes were studied in 16 developmental stages. Their expression profiles are presented in Fig. 1. This divides the genes into three main groups. Briefly, our results show that genes coding for dishevelled, GSK-3 , p53, VegT,

Vg1 and Xnot were already expressed maternally and are present in the first stages of the development, whereas genes coding for activin, chordin, derriere, follistatin, HNF-3beta, N-CAM and siamois are zygotic and expressed after the mid-blastula stage. Xbra and cerberus have maximum expression at the mid-blastula stage. This approach allowed the individual *X. laevis* developmental stages and their phenotype to be connected with the expression levels of individual examined genes (3). The connections detected by this quantitative approach were found to be in good agreement with either previous data obtained in other studies and by different techniques or with what has been expected to take place during the development.

Profiles of expression of Src, Fyn, Yes and Csk tyrosine kinases throughout *X. laevis* developmental stages 1-45 were determined and coincidence of the profiles of individual tyrosine kinases with onset of phenotypic changes characterizing the individual stages of development is being analysed.

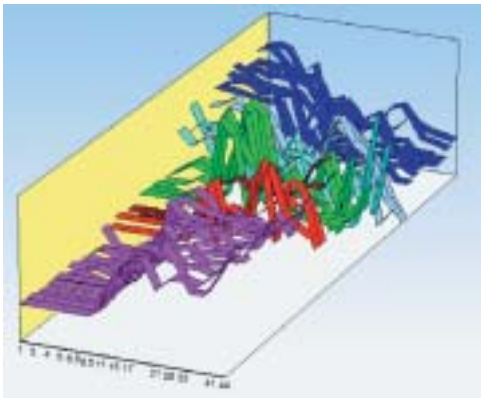


Fig.1. Expression of *Xenopus laevis* developmental genes during development (stages 1–44). Maternal genes are shown in blue (dishevelled, p53, VegT, and Xnot), cyan (Vg1) and sky blue (GSK-3), MBT (mid-blastula transition) genes are shown in green (Xbra and cerberus), and the late genes are shown in pink (activin, chordin, derriere, follistatin, goosecoid, HNF-3beta, and siamois) and in red (N-CAM). In some cases two to four expression profiles of the same gene are presented.

Mouse *src* gene intron 5 - a new taxonomic marker of mouse species and subspecies

We found that a unique strain- and species-specific polymorphism of a single compound microsatellite region, designated D2Img1, about 260 nucleotides long, has a potential to distinguish all 15 mouse species and subgenera representing major evolutionary branches from Europe, Asia and Africa, as well as certain inbred lines of laboratory mouse. The 260 nt fragment could be produced from all tested mouse species using a universal pair of primers. The rationale behind this potential revealed reconstruction of a phylogenetic history of the mouse from the microsatellite data set using a parsimony analysis. The family tree obtained is very similar to canonical trees constructed from other combined data indicating a close relationship between microsatellite polymorphism and mouse phylogeny (1).

We believe that the microsatellite would be appreciated by all mouse biologists as an independent marker on the second chromosome for genotyping of individual mouse species, as well as by researchers interested in molecular evolution of microsatellites. This is for the first time that a single microsatellite has such a high discrimination potential.

Ad (ii) **Gene expression in bacteria**

Bacterial transcription - promoter structure, strength and regulation in Gram positive bacteria (*B. subtilis*). Model system: rRNA expression regulation.

We studied the regulation of ribosome synthesis at the level of initiation of transcription of ribosomal RNA (rRNA) in *Bacillus subtilis*. Current models of this process have been derived mostly from studies with *Escherichia coli*. Our work demonstrates that each organism has solved

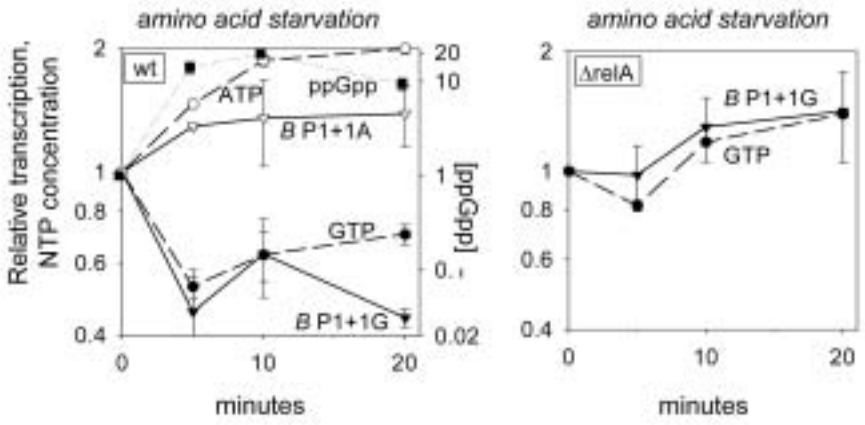


Fig. 2C, D. Amino acid starvation was induced at time 0. Promoter activity was followed by quantitative primer extension of specific transcripts. ATP, GTP, ppGpp levels at corresponding time points are indicated.

the requirements for proper rRNA promoter strength and regulation in its own unique manner. Thus, it seems that at least two models of regulation of this key cellular process exist among bacteria.

Fig. 2C, D shows one selected aspect of the regulation that differs between the two organisms: the effects of ppGpp on rRNA promoter regulation when amino acid starvation is induced.

In *E. coli*, in this situation, increasing ppGpp levels directly inhibit rRNA promoter activity. Figs. 2C, D show that ppGpp acts indirectly in *B. subtilis.* In this organism, rRNA promoter activity depends on intracellular levels of GTP, the initiating nucleotide of rRNA promoters (BP1+1G). A mutant BP1+1A rRNA promoter engineered to initiate with ATP increases its activity in this situation, following the increasing ATP levels (Fig. 2C). Its inability to be inhibited by increasing ppGpp levels suggests that ppGpp may have an indirect role.

Fig. 2D shows that the *relA* (RelA synthetizes ppGpp in response to aa starvation) strain is unable to shut down the synthesis of rRNA due to the unchanged level of GTP and, consequently, rRNA promoter activity.

It was shown that ppGpp directly affects GTP synthesis by inhibiting one of the enzymes required for its synthesis. Therefore, it seems that the effect of ppGpp on rRNA promoters is accomplished by regulating the levels of GTP (2).

The ***ybx*F gene** was found to extend the highly conserved *str* operon in Gram+ *B. stearothermophilus* and *B. subtilis* upstream of *rps*L, *rps*G, *fus* and *tuf* housekeeping genes coding for ribosomal proteins S12 and S7 and proteosynthetic elongation factors EF-G and EF-Tu, respectively. In contrast to all other genes of the operon, the *ybx*F gene does not seem to be an essential gene in *B. subtilis* because we were not able to detect any differences between wild-type and *ybx*F knockout bacteria.

Ad (iii) **Structure-function relationships in proteins: protein domains and protein functions and thermostability**

Model system: bacterial elongation factors EF-Tu

All elongation factors EF-Tu are monomeric GTPases composed of a ~400 amino acid residue long polypeptide chain folded into three clearly distinct domains. They deliver aminoacyl-tRNAs

to the ribosomal A-site during mRNA translation process. Their N-terminal domain 1 or G-domain (~200 residues) is the site of GDP/GTP binding and of GTPase activity. It is called a catalytic domain and has an / structure. The middle domain 2 and the C-terminal domain 3 (each composed of ~100 residues) are barrels. Domains 2+3 have no known binding or catalytic activity. Except for the fact that all three domains are absolutely required for the attachment of aminoacyl-tRNA to the EF-Tu molecule in response to bound GTP during translation, the reasons for the domain arrangement of EF-Tus are not fully understood. The high degree of sequence homology also predetermines EF-Tu proteins for studies of structural features of adaptation of multidomain proteins to different living conditions.

In the present study we assessed the contribution of individual domains of EF-Tu to the binding of guanine nucleotides GDP and GTP, to the intrinsic GTPase activity and to the thermal stability of EF-Tu structure and functions. We compared EF-Tu domains from two different organisms: mesophilic, Gram- *E. coli* (Ec, growing at 37°C), and thermophilic, Gram+ *B. stearothermophilus* (Bst, growing at 58 °C). We examined isolated G-domains, full-length EF-Tus and six Ec-Bst chimeric forms of EF-Tu formed by combinations of domains of both EF-Tus. Our experiments provide evidence for similar, different and even opposite roles of the domains of the two EF-Tus in the accomplishment of EF-Tu functions (4,5).

Thermostability: The G-domains appear to set up a "basic" level of the thermostability of either EF-Tu. It is ~20 °C higher with BstG-domain than with EcG-domain; this correlates with a higher content of charged amino acid residues at the expense of polar uncharged residues and a higher volume of the hydrophobic core in Bst G-domain in comparison to Ec G-domain. These differences very likely explain the higher BstEF-Tu thermostability in comparison to EcEF-Tu. The domains 2+3 (mainly domains 3) of both EF-Tus increase the thermostability of G-domain by stabilization of its helical regions and, in turn, this coincides with the stabilization of G-domains' functions, such as GDP/GTP binding and GTPase activity, up to the level of the growth temperature optimum of either organism. The stabilizing effect by Ecdomains 2+3 is higher in GDP conformation than in GTP conformation.

GDP/GTP binding activity: Ecdomains 2+3 and Bstdomains 2+3 participate principally differently in building the GDP/GTP binding activity of respective EF-Tus. Whereas in EcEF-Tu, domains 2+3 are absolutely required to achieve its physiological (i.e. high and differential) affinity for GDP and GTP, in BstEF-Tu the high and differential affinity for GDP and GTP (which is in the same range as that of EcEF-Tu) appears to be the function of the G-domain itself. It is not influenced by the presence or absence of domains 2+3.

GTPase activity: Isolated G-domains of both EF-Tus displayed similar GTPase activity at their optimal temperature. However, non-catalytic domains 2+3 influenced the activity oppositely. Ecdomains 2+3 (mainly domain 2) suppressed the GTPase activity of EcGdomain (about 2.5 times) whereas Bstdomains 2+3 (mainly domain 2) stimulated the BstG-domain GTPase (about 3 times) (Fig. 3).

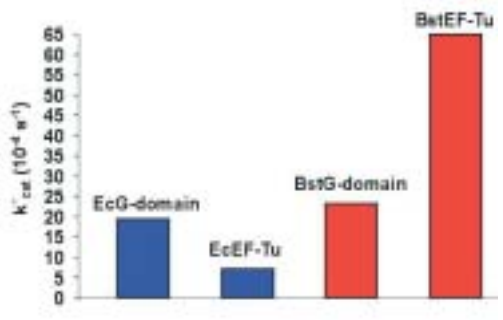


Fig 3. GTPase activity of EcEF-Tu, BstEF-Tu and their G-domains. Opposite effects of *Ec*domains 2+3 and *Bst*domains 2+3.

The results imply that despite the same function and chemistry of all examined EF-Tu proteins and a high degree of identity in their sequences, in EcEF-Tu and BstEF-Tu, different G-domain structural and G-domain-domains 2+3 interaction strategies were developed to establish the same GDP/GTP binding and GTPase phenotype.

These findings together with the results of transcription studies on *B. subtilis* rRNA promoters described above extend the list of differences between Gram+ and Gram– bacteria at the level of gene expression regulatory mechanisms.

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 301/02/0408, 303/02/0689, 204/05/P510, 204/05/H023), and by the Grant Agency of the Academy of Sciences of the Czech Republic (research grants Nos. A 505 2206, B 5005 20503).

Publications

1. Fučík, V., Šindelka, R., Beran, J., Ferjentsik, Z., Flégr, J., Jonák, J.: Microsatellite polymorphism in the *src* gene intron 5 characterizes the main branches of the mouse evolutionary tree. *Mol. Phylogenet. Evol., submitted.*

2. Krásný, L., Gourse, R.L.: An alternative strategy for bacterial ribosome synthesis: *Bacillus subtilis* rRNA transcription regulation**.** *EMBO J. 23: 4473-4483, 2004.*

3. Kubista, M., Andrade, J.M., Bengtsson, M., Forootan, A., Jonák, J., Lind, K., Šindelka, R., Sjöback R., Sjögreen B., Strömbom L., Stahlberg A., Zoric N.: The real-time polymerase chain reaction. *Mol. Aspects Med. 27: 95-125, 2006.*

4. Šanderová, H., Hůlková, M., Maloň, P., Kepková, M., Jonák, J.: Thermostability of multidomain proteins: Elongation factors EF-Tu from *Escherichia coli* and *Bacillus stearothermophilus* and their chimeric forms. *Protein Sci. 13: 89-99, 2004.*

5. Šanderová, H., Jonák, J.: Opposite roles of domains 2+3 of *Escherichia coli* EF-Tu and *Bacillus stearothermophilus* EF-Tu in the regulation of EF-Tu GTPase activity. *Biochim. Biophys. Acta 1752: 11-17, 2005.*

6. Šindelka, R., Ferjentsik, Z., Jonák, J.: Developmental expression profiles of *Xenopus laevis* reference genes. *Dev. Dyn. 235: 754-758, 2006.*

In the year 2005 the Department co-organized a **FEBS Practical Course: Real-time Quantitative RT-PCR, Analysis of Gene Expression** (Prague, 18-23 September 2005)**.**
(see the poster and http://www.img.cas.cz/ge).

Every year a 14-day course **„Advances in Molecular Biology and Genetics“** is organized by the Department for PhD students in biomedicine (http://www.kav.cas.cz/pdsb) and every other year a new text-book with lectures is provided [Group of authors: **Molecular biology and genetics XI** (Jonák, J., Jr. and Jonák, J., ed.), Institute of Molecular Genetics AS CR, Prague 2004, 210 pp (in Czech)].

Micromorphology of Biopolymers

Head: Jan Korb

Research Fellows: Jitka Štokrová, Josef Říman

Undergraduate Student: Vojtěch Žíla (part time)

Technicians: Ivana Lišková, Jiřina Musilová

Introduction

In the last two years the research in the laboratory was mainly focused on the following topics:

(i) Nucleolar architecture and interaction of cellular proteins with viral antigens were studied in the cells infected with mouse polyomavirus (PyV). We have focused on the analysis of nuclear structure and processes taking place in the later phases of viral infection. Besides, model chimeric pseudocapsids (VLPs) based on mouse PyV, carrying EGFP protein fused with the G-terminal part of the PyV VP3 minor capsid protein were investigated.

(ii) Morphological analysis of colorectal adenocarcinoma cell line HT29 after treatment with sodium butyrate and glucose deprivation was performed. Alterations in mitochondria function and morphology in HT29 cells after treatment with agents inducing differentiation and apoptosis were analysed.

(iii) The topography of signalling molecules in cells activated through Fc RI dimers on rat basophilic leukaemia (RBL) cells was analysed in collaboration.

Results

Model chimeric pseudocapsids (VLPs), based on murine polyomavirus (PyV), carrying EGFP protein fused with the C-terminal part of the PyV VP3 minor capsid protein, were investigated. We proved their entry not only into mouse fibroblasts and epithelial cells, but also into human and mouse dendritic cells (DC) *in vitro*. Similarly as in fibroblasts and epithelial cells the VLPs were present in early endosomes during further trafficking in DC and later they were found in endoplasmic reticulum. Unfolding of VLPs was observed before they reach the perinuclear space. Although VLPs were degraded by both lysosomes and proteasomes in all cell types investigated, they surprisingly did not induce upregulation of DC co-stimulation molecules or maturation markers *in vitro.* However, VLPs induced interleukin 12 secretion in DC. Analysis of the behaviour of major structural PyV protein VP1, with a point mutation in the calcium-binding pocket, surprisingly showed that calcium is not necessary for VP1 capsid assembly. However, the mutation alters the stability and surface conformation of VLPs and therefore also their interactions with cellular components.

Further, we have found that PyV virions are internalized not only by caveolar endocytosis, but that they are able to utilize a non-caveolin and non-clathrin endocytic pathway. Entry of PyV is connected with tyrosine-kinase activation. Vesicles containing both caveolin and virions fuse with EEA1-positive early endosomes during their transport through the cytoplasm. Productive infection is dependent on acidic pH of endosomes. PyV is further transported through recycling endosomes to endoplasmic reticulum. Virions of PyV use both tubulin and actin cytoskeleton for their movement in the cytoplasm (2,4 5).

Four clones of the parental HT29 adenocarcinoma cell line were ultrastructurally characterized and their response to the treatment with sodium butyrate, as the differentiation-inducing agent, was analysed. The results revealed that the parental cell line is heterogenous and that this line consists of cells that have a potential to differentiate into distinct cell types as do cells of the normal intestinal epithelium. The cells of two clones (H8 and G9) were changed by induced differentiation into an absorptive type, while the cells of other two clones (G10 and A3) resembled goblet-like cells after butyrate treatment. It is obvious that HT29 cells contain the same information as the multipotential stem cells of normal intestinal crypts (3, 6).

The distribution of Fc RI subunit, Grb2 and Syk was analysed by immunoelectron microscopy on isolated membrane sheets from both resting and activated RBL cells. Fc RI in resting cells was found homogenously distributed as singlets or small clusters as well as in 5.14-activated cells, where it was occasionally associated with Syk. In contrast, Ag-mediated aggregation of Fc RI resulted in accumulation of Fc RI subunits in osmiophilic regions of the plasma membrane together with Syk and Grb2. Quantitative analysis of gold particle clustering confirmed that only Ag-activated cells exhibited enhanced formation of Fc RI clusters with inceased co-localization of Fc RI and Grb2. These data demonstrate that full secretory response is attained in cells activated through Fc RI dimers in the absence of formation of large clusters of Fc RI, Grb2 and Syk in osmiophilic membranes (1).

The members of the Micromorphology of Biopolymers group also participated as collaborators in other projects (7).

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 204/03/0593, 301/03/0596), by the Grant Agency of the Academy of Sciences No. IAA 5052310 and Howard Hughes Medical Institute Grant No. 75195-540501.

Publications

1. Dráberová, L., Lebduška, P., Hálová, I., Tolar, P., Štokrová, J., Tolarová, H., Korb, J., Dráber, P.: Signaling assemblies formed in mast cells activated via Fc receptor dimers. *Eur. J. Immunol. 34: 2209-2219, 2004.*

2. Adamec, T., Palková, Z., Velková, K., Štokrová, J., Forstová, J.: Point mutation in calcium-binding domain of mouse polyomavirus VP1 protein does not prevent virus-like particle formation, but changes VP1 interactions with *Saccharomyces cerevisiae* cell structures. *FEMS Yeast Res. 5: 331-340, 2005.*

3. Štokrová, J., Sovová, V., Šloncová, E., Kučerová, D., Tuháčková, Z., Korb, J.: Response of HT115, a highly invasive human colorectal adenocarcinoma cell line, to sodium butyrate treatment and glucose deprivation. *Int. J. Oncol. 26: 793-799, 2005.*

4. Bouřa, E., Liebl, D., Spíšek, R., Frič, J., Marek, M., Štokrová, J., Holáň, V., Forstová, J.: Polyomavirus EGFP-pseudocapsids: Analysis of model particles for introduction of proteins and peptides into mammalian cells. *FEBS Lett. 579: 6549-6558, 2005.*

5. Liebl, D., Difato, F., Horníková, L., Mannová, P., Štokrová, J., Forstová, J.: Mouse polyomavirus enters early endosomes, requires their acidic pH for the productive infection and meets transferrin cargo in Rab11-positive endosomes. *J. Virol., in press.*

6. Štokrová, J., Šloncová, E., Sovová, V., Kučerová, D., Žíla, V., Turečková, J., Vojtěchová, M., Korb, J., Tuháčková, Z.: Characterization of four clones derived from human adenocarcinoma cell line, HT29, and analysis of their response to sodium butyrate. *Int. J. Oncol. 28: 559-565, 2006.*

7. Bauerová-Zábranská, H., Štokrová, J., Stříšovský, K., Hunter, E., Ruml, T., Pichová, I.: The RNA binding G-patch domain in retroviral protease is important for infectivity and D-type morphogenesis of Mason-Pfizer monkey virus. *J. Biol. Chem. 280: 42106-42112, 2005.*

Recombinant Expression and Structural Biology

Head: Juraj Sedláček

Research Fellows: Jiří Brynda, Milan Fábry, Magda Hořejší, František Kaprálek (until 2005), Jan Ondráček, Pavlína Řezáčová (absent on leave from 2005), Renata Štouračová (absent on leave from 2003), Bronislava Uhnáková (from 2005),Vladimír Tomášek (part time)

Graduate Students: Vlastimil Král[1], Pavel Mader[1], Veronika Suchá-Krejčíříková

Research Assistants: Irena Sieglová, David Kredba (part time)

Undergraduate Students: Vanda Bartoňová (until 2005)

Technicians: Jitka Káralová, Jitka Kredbová, Věra Mrkvičková

Secretary / Supply Manager: Jaroslava Zinková

[1] Supported from grant FD-K3/001, Ministry of Industry and Commerce of the Czech Republic

Introduction

Structure and mechanism studies of non-active-site inhibitors of HIV protease (HIV PR) are long-term activities of this group. The retroviral proteinase of HIV is an attractive target for antiviral intervention. Our earlier studies have shown potent (nanomolar-Ki) inhibition of HIV PR by monoclonal antibodies (mAbs) upon their binding to the top-of-flap region or to the N-terminus of the HIV PR molecule. On the basis of X-ray structural results, a distortion of the top-of-flap loop and dissociation of HIV PR monomers have been proposed as the respective mechanisms of the inhibition. An underlying idea of the search for non-active-site inhibitors of HIV PR is development of inhibitors that would provide potent action also towards the HIV PR mutants displaying single or multiple resistances to canonical (active-site) inhibitors or drugs.

The group is also involved in another way to overcome the problem of drug resistance of HIV PR, i.e. an extensive X-ray structural work is performed on the complexes of novel HIV PR active-site inhibitors developed at the Institute of Organic Chemistry and Biochemistry (ref. Rinnová et al., ABB 2000, *382*, 22-30; 1,2).

The Department has well equipped biochemical, molecular cloning and protein purification and crystallization laboratories. Its experienced staff operates an X-ray diffraction station (as the first facility of this type on the national scale) enabling them to perform standard in-house protein crystallography. Also the computational support includes up-to-date hardware and software.

The research programme of the group has diversified, *inter alia*, into study of the structural basis of other protein-protein or protein-sugar interactions, and into practically oriented protein design.

Results

HIV protease (PR) represents a prime target for rational drug design, and protease inhibitors

(PI) are powerful antiviral drugs. Most of the current PIs are pseudopeptide compounds with limited bioavailability and stability, and their use is compromised by high costs, side effects, and development of resistant strains.

In a search for novel PI structures, a group of inorganic compounds, icosahedral metallacarboranes, were identified as candidates for a novel class of non-peptidic PIs (1). Potent, specific, and selective competitive inhibition of HIV PR by substituted metallacarboranes, such as sodium hydrogen butylimino bis-8,8-[5-(3-oxa-pentoxy)-3-cobalt bis(1,2-dicarbollide)]di-ate, was reported. The structure of the parent cobalt bis(1,2-dicarbollide) (compound I in Fig. 1) in complex with HIV PR was determined at 2.15 Å resolution by protein crystallography and represents the first carborane-protein complex structure determined. It shows the following mode of PR inhibition: two molecules of the parent compound bind to the hydrophobic pockets in the flap-proximal region of the S3 and S3 subsites of PR. We therefore suggest that these compounds block flap closure in addition to filling the corresponding binding pockets as conventional PIs. This type of binding and inhibition, chemical and biological stability, low toxicity, and the possibility to introduce various modifications make boron clusters attractive pharmacophores for potent and specific enzyme inhibition.

Details of interactions of the monoclonal antibody 1696, elicited by HIV-1 protease, that inhibits the activity of both HIV-1 and HIV-2 proteases with inhibition constants in the low nanomolar range were determined (3). The antibody cross-reacts with peptides derived from the N-terminal region of both proteases. The crystal structure of the recombinant single-chain Fv fragment of 1696 complexed with an N-terminal peptide from the HIV-2 protease has been determined at 1.88 Å resolution. Interactions of the peptide with scFv1696 are compared with the previously reported structure of scFv1696 in complex with the corresponding peptide from HIV-1 protease, and the origin of cross-reactivity of mAb1696 with HIV proteases is explained.

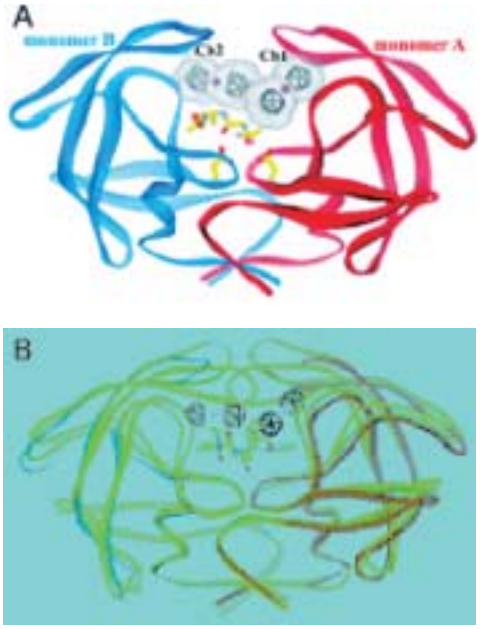


Fig. 1. X-ray structure analysis of the binding of compound 1 to HIV-PR.

(*A*) Overall structure of the HIV PR-compound 1 complex. The PR dimer is in ribbon representation with the two catalytic aspartates shown in sticks. Two compound 1 molecules are represented by their van der Waals surfaces and grey stick model, with cobalt ions shown as magenta spheres. The autoproteolytic peptide product is represented as a stick model.

(*B*) Superposition of PR-compound 1 complex with PR-lopinavir complex and with the free PR structure. Protease complex with lopinavir (PDB ID code 1MUI) is represented in yellow ribbons, lopinavir is shown as a stick model, free PR structure (PDB ID code 1HHP) is shown in green ribbons, and colour coding for PR-compound 1 complex is the same as in *A*.

In the course of investigating the complex of HIV-1 protease with a novel phenylnorstatine inhibitor, two inhibitor molecules were found to be bound to the enzyme, one in non-productive mode. All quantitative parameters found matched criteria of supplemental ordering of the crystal with the interface ligand binding (4). It is generally believed that the resolution limit is determined by the order of the crystal structure; a denser and highly ordered crystal will have a high resolution limit. In our work, contribution of the density of packing to the crystal quality became apparent with a correlation done for 55 structures of HIV PR complexes of orthogonal symmetry. The plot of the calculated „per HIV PR molecule volumes" and the stated resolution limits (Fig. 2) displays a direct proportionality trend for this group, though with a mediocre correlation coefficient.

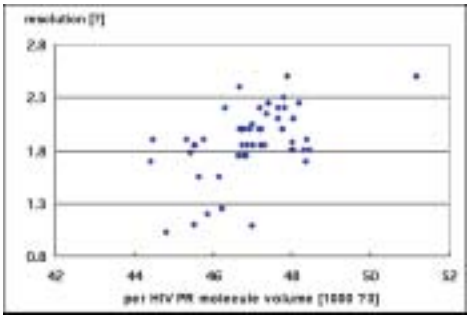


Fig. 2. Plot of the calculated „per HIV PR molecule volumes" and the stated resolution limits for 55 PDB entries. For details see the text.

In qualitative terms, this correlation can be understood so that crystal quality is improved with denser crystal packing since the protein molecules are less exposed to the solvent and their side chains more restricted in movement.

The HipHop refinement procedure, the development of which we started more than two years ago, results in the generation of typically ten equally accurate models that differ in details of side- and/or main-chain conformations and the total number (and position) of water molecules added. With an earlier version of the HipHop algorithm, we were already able to better interpret features of a high-resolution protein structure that would otherwise have stayed disguised (5). The method has been further developed since, and more information regarding the programme can be found at http://www.img.cas.cz/hiphop, from where the software can also be obtained.

The activities within the "Center for Molecular and Gene Biotechnology" and „Center for Targeted Therapeutics" represented applied research. Recombinant viral antigens developed in the group became a component of tested diagnostic kits for antibodies against cytomegalovirus. Recombinant formats of antibodies potentially useful for practical diagnostic or therapeutic purposes are being developed.

The international collaboration of this group was supported from the C.E.C. 5th Program, Concerted Action QLK2-CT-2001-02360. Local support included research grants from the Grant Agency of the Czech Republic, Nos. 203/02/0405 and 203/05/2141; and from the Ministry of Industry and Commerce of the Czech Republic, FD-K3/001. The participation in the "Center for Molecular and Gene Biotechnology" and the „Center for Targeted Therapeutics" was supported from the Ministry of Education, Youth and Sports of the Czech Republic under codes LN00B030 and IM4635608802, respectively.

Publications

1. Cígler, P., Kožíšek, M., Řezáčová, P., Brynda, J., Otwinowski, Z., Pokorná, J., Plešek, J., Grüner, B., Dolečková-Marešová, L., Máša, M., Sedláček, J., Bodem, J., Kraeusslich, H.-G., Král, V., Konvalinka, J.: From non-peptide towards non-carbon protease inhibitors: metallacarboranes as specific and potent inhibitors of HIV protease. *Proc. Natl. Acad. Sci. USA 102: 15394-15399, 2005.*

2. Petroková, H., Dušková, J., Dohnálek, J., Skálová, T., Vondráčková-Buchtelová, E., Souček, M., Konvalinka, J., Brynda, J., Fábry, M., Sedláček, J., Hašek, J.: Role of hydroxyl group and R/S configuration of isostere in binding properties of HIV-1 protease inhibitors. *Eur. J. Biochem. 271: 4451-4461, 2004.*

3. Řezáčová, P., Brynda, J., Lescar, J., Fábry, M., Hořejší, M., Sieglová, I., Sedláček, J., Bentley, G.A.: Crystal structure of a cross-reaction complex between an anti-HIV-1 protease antibody and an HIV-2 protease peptide. *J. Struct. Biol. 149: 332-337, 2005.*

4. Brynda, J., Řezáčová, P., Fábry, M., Hořejší, M., Štouračová, R., Souček, M., Hradílek, M., Konvalinka, J., Sedláček, J.: Inhibitor binding at the protein interface in crystals of a HIV-1 protease complex. *Acta Crystallogr. D Biol. Crystallogr. 60: 1943-1948, 2004.*

5. Ondráček, J., Weiss, M.S., Brynda, J., Fiala, J., Jursík, F., Řezáčová, P., Jenner, L.B., Sedláček, J.: Regular arrangement of periodates bound to lysozyme. *Acta Crystallogr. D Biol. Crystallogr. 61: 1181-1189, 2005.*

Biology and Biochemistry of Fertilization

Head: Jana Pěknicová

Research Fellows: Jana Čapková, Vendula Kyselová (until April 2005)

Graduate Students: Pavel Koubek, Alena Sedláková[1]

Research Assistants: Fatima Elzeinová, Hasmik Margaryan, Alena Kubátová

Undergraduate Students: Marie Pexidrová, (graduated 2004), Helena Líbalová (from September 2005), Petr Páral (from September 2005)

Technician: Věra Doušová

[1]Supported by Faculty of Science, Charles University, Prague

Introduction

Our long-term research programme concentrates on studying the molecular mechanisms of fertilization. The sperm-egg interactions in mammals consist of a series of specialized and regulated events that initially involve egg-induced activation of the spermatozoon and ultimately result in sperm-induced activation of the egg. Sperm and oocyte proteins (zona pellucida) participate in the highly specialized interaction between gametes.

Many chemicals released into the environment can disrupt the endocrine function in animals and human and have a negative effect on fertility. This includes chemicals that occur naturally in plants such as phytoestrogens, and also man-made chemicals (xenoestrogens).

Ankylosing enthesopathy (ANKENT) is a naturally occurring joint disease in laboratory mice with progressive stiffening of the ankle and tarsal joints of the hind paws. For a number of features ANKENT appears analogous to the human spondylarthropathies (SpA). ANKENT might therefore be used as a model of human disease ankylosing spondylitis (AS). Relative risk factors for ANKENT and AS are known. The dependence of ANKENT on gender, particularly on male hormone testosterone, was investigated.

In the last two years the study of our group was focused on three topics:

1. Traditional study of cell surface and intra-acrosomal sperm proteins playing a role in the primary and secondary sperm-zona pellucida binding process during fertilization.

2. Effect of endocrine disruptors on mammalian fertility. Selected endocrine disruptors (present as food contaminants constituting reproductive toxicology risk) were tested for their influence on *in vivo* fertility of outbred lines of mice. A panel of monoclonal antibodies against acrosomal sperm proteins detected the occurrence of sperm damage. Monoclonal antibodies against intra-acrosomal sperm proteins were used as suitable and sensitive markers for detection of spermatogenesis in human medicine.

3. Role of male hormone testosterone in ANKENT occurrence and significance of intestinal inflammation for ANKENT development.

Results

1. Monoclonal antibodies against human intra-acrosomal proteins were used for evaluation of human sperm pathology (2-5) and detection of spermatids in ejaculates of men with azoospermia (ejaculate without spermatozoa). Our antibodies can bind to acrosomal proteins in round spermatids and can prove the spermatogenesis. The spermatids or spermatozoa obtained from testes by biopsy can be utilized in assisted reproduction (6).

The spermatozoa with varied semen characteristics (oligozoospermia, oligoasthenoteratozoospermia, and MESA native and frozen/thawed, TESE native and frozen/thawed) were tested with anti-acrosomal antibodies for detection of the sperm acrosome, and fertilization, transfer, pregnancy and implantation rates were recorded in each category.

The fertilization rate and statistical evaluation showed differences between morphologically normal and pathological sperm and other groups. The freezing-thawing procedure had no influence on the fertilization of testicular sperm but epididymal frozen/thawed sperm had a higher fertilization rate. The spermatozoa with varied semen characteristics and good quality, also detected with specific antibodies, gave the best fertilization rates. The paternal effect has not been proved in other parameters (7).

2. The effect of selected endocrine disruptors, xenoestrogen (diethylstilbestrol) and phytoestrogen (genistein) on the integrated reproductive process as well as on individual reproductive organs and gametes in the CD1 mice in a multigenerational study was tested. Diethylstilbestrol had a negative influence on the number of born offspring of mice, on reproductive organs and on the acrosome integrity of mouse spermatozoa. Contrary to that, treatment by phytoestrogen had not affected the body weight and other body parameters, the level of serum hormones and *in vivo* fertility. Application of monoclonal antibodies against intra-acrosomal proteins as a tool for testing sperm damage is a unique method in these studies (1).

The *in vitro* biological activity of methanolic and aqueous extracts from *Lepidium meyenii* Walp. (Brassicaceae) dehydrated hypocotyls were studied. Analysis of lipid compounds revealed important quantities of steroids in the methanol and in the aqueous extract, mainly -sitosterol. In MCF-7 cell line, both extracts have shown oestrogenic activity comparable with that of silymarin. From a comparison of the maca extracts with 17 -oestradiol we conclude that the oestrogenic activity is exhibited in the range 100 to 200 g/ml. This activity is at least in part due to their steroids and may contribute to maca *in vivo* activity on spermatogenesis and potency as is known from the literature and from traditional medicine (8).

3. The role of sexual dimorphism and testosterone was studied in non-castrated, castrated and testosterone-substituted castrated male mice and in control and testosterone-treated female mice. ANKENT occurred only in non-castrated and castrated males injected with testosterone. Females injected with testosterone did not develop ANKENT. Thus sexual dimorphism, but not testosterone itself is responsible for ANKENT development (10).

On the ANKENT model, a possible relationship between gut inflammation and joint disease was investigated. Clinical symptoms of chronic colitis confirmed the inflammatory process in the colon in dextran sodium sulphate-treated mice, but no significant difference in ANKENT frequency was found between mice with colitis and control healthy mice without DSS treatment. ANKENT could not be triggered solely by chemically induced inflammatory process in gut (9).

The HLA-B27 protein was purified from blood lymphocytes of AS patients and healthy control, and pool sequencing of the bound peptides was performed. The frequency of Glu was increased and Gln was decreased in peptides eluted from AS patients in comparison with the healthy control (11,12).

The group was supported by the grants Nos. 204/02/1373, 305/03/0287 and 524/03/0178 from the Grand Agency of the Czech Republic and grants Nos. NJ/6463-3, NJ/7463-3 from the Grant Agency of the Ministry of Health of the Czech Republic.

Publications

1. Kyselová, V., Pěknicová, J., Boubelík, M., Buckiová, D.: Body and organ weight, sperm acrosomal status and reproduction after genistein and diethylstilbestrol treatment of CD1 mice in a multgenerational study. *Theriogenol. 61: 1307-1325, 2004.*

2. Teplá, O., Pěknicová, J.: Treatment of germ cells *in vitro*. *Postgraduální medicína 6: 222-224, 2004* (in Czech).

3. Pavlásek, J., Pěknicová, J., Ulčová-Gallová, Z., Nováková, P., Reischig, J., Mičanová, Z., Rokyta, Z.: Significance of the acrosomal proteins and spermagglutinating antibodies detection in human reproduction. *Czech Gynaecol. 69: 306-311, 2004.*

4. Baracova, V., Mollova, M., Stamenova, M., Ivanova, M., Pěknicová, J.: Identification and isolation of boar sperm specific antigens with potential role in sperm – egg interaction. *J. Reprod. Immunol. 64: 91-106, 2004.*

5. Kraus, M., Tichá, M., Železná, B., Pěknicová, J., Jonáková, V.: Characterization of human seminal plasma proteins homologous to boar AQN spermadhesins. *J. Reprod. Immunol. 65: 33-46, 2005.*

6. Pěknicová, J., Chládek, D., Hozák, P.: Monoclonal antibodies against sperm intra-acrosomal antigens as marker for male infertility diagnostics and estimation of spermatogenesis. *Am. J. Reprod. Immunol. 53: 42-49, 2005.*

7. Teplá, O., Pěknicová, J., Kočí, K., Mrázek, M., Elzeinová, F.: Evaluation of the fertilization rate after intracytoplasmic sperm injection with varied semen characteristics of the human spermatozoa tested by intra-acrosomal antibodies. *Fertil. Steril., in press.*

8. Valentová, K., Buckiová, D., Křen, V., Pěknicová, J., Ulrichová, J., Šimánek, V.: The *in vitro* biological activity of Lepidium meyenii extracts. *Cell Biol Toxicol. 22: 91-99, 2006.*

9. Čapková, J., Štěpánková, R., Hudcovic, T., Šinkora, J., Řeháková, Z.: Experimental colitis does not increase the prevalence of ANKENT, a spontaneous joint disease in mice. *Folia Microbiol. 49: 745-750, 2004.*

10. Čapková, J., Ivanyi, P., Řeháková, Z.: Sexual dimorphism, but not testosterone *per se,* is responsible for ankylosing enthesitis of ankle in B10.BR (H-2^k) males. *Ann. Rheum. Dis. 65: 130-132, 2006.*

11. Stodůlková, E., Man, P., Pohl, J., Van Nguyen, D., Vaingátová, S., Ivašková, E., Pla, M., Čapková, J., Sedláčková, M., Ivanyi, P., Flieger, M.: Peptides eluted from HLA-B27 of human splenocytes and blood cells reveal a similar but partially different profile compared to *in vitro* grown cell lines. *Immunol. Lett. 94: 261-265, 2004.*

12. Stodůlková, E., Pohl, J., Man, P., Votruba, J., Čapková, J., Sedláčková, M., Vašková, E., Ivany, P., Flieger, M.: Comparison of amino acid compositions of peptides eluted from HLA-B27 molecules of healthy individuals and patiens with ankylosing spondylitis. *Immunol. Lett. 103: 135-141, 2006.*

Biochemistry of Reproduction

Head: Věra Jonáková

Research Fellows: Pavla Maňásková, Blanka Železná, Marek Kraus (until January 2005), Leopold Veselský (40%, until December 2004), Dana Čechová (20%, until December 2004)

Graduate Students: [1]Eva Cibulková (from November 2005), Andriy Dorosh

Undergraduate Students: Dagmar Hýblová (graduated 2004), Eva Cibulková (graduated 2005), Nina Davidová

Technicians: Magda Hošková (60%), Jitka Jelínková

[1] Supported by Faculty of Science, Charles University, Prague

Introduction

Mammalian fertilization is a unique event in which morphologically disparate gametes recognize, bind and fuse with each other. This event includes highly regulated biochemical interactions: binding of seminal plasma proteins to the sperm surface during ejaculation, interaction of sperm surface proteins with oviductal epithelial cells, sperm capacitation, gamete recognition, primary and secondary binding of the sperm to the ovum, acrosome reaction of sperm, penetration of the sperm through zona pellucida (ZP) of the ovum and gamete fusion.

Seminal plasma is a mixture of secretory products of the male reproductive organs. Boar seminal plasma contains mainly proteins with a characteristic CUB domain, the spermadhesin family (AQN, AWN, PSP proteins) belonging to a large family of developmentally regulated proteins, and DQH sperm surface protein, which has different protein structure, contains two fibronectin type II domains and is associated with the large family of cell and matrix adhesion proteins. These proteins bind to the sperm surface membrane during ejaculation and form the protein-coating layers. Sperm surface proteins participate in various types of interactions in further steps of the reproduction process.

Results

The binding properties of proteins isolated from boar seminal plasma and their role in the reproduction process are discussed. Seminal plasma proteins (AQN, AWN, PSP, DQH) and their aggregated forms (fractions I-III) interact with phosphorylcholine-containing components of the sperm membrane during ejaculation. The interaction with phospholipid components is responsible for protein adsorption to the sperm surface membrane. Sperms coated with proteins participate in various types of interactions in the female oviduct during reproduction. Saccharide-based interactions of sperm surface proteins play a role in the binding of sperm to oviductal epithelium, in sperm capacitation and in primary binding of sperm to ZP. Mutual interactions between seminal plasma proteins participate in the arrangement and remodelling of sperm-coating layers. Various methods are applied to show the saccharide-binding ability of sperm surface proteins or to study different factors affecting this interaction (1).

The key proteinase in the fertilization process is acrosin, localized in the acrosome of the spermatozoon as proacrosin. Acrosin is involved in the acrosome reaction of spermatozoa, in the secondary binding of the sperm to ZP and penetration of the ZP by sperm. The biological role of acrosin inhibitors in sexual fluids is to inactivate the prematurely released active form of acrosin from occasionally damaged spermatozoa and thus to protect the male and the female genital tract against proteolytic degradation. Spermadhesins attached to the sperm head serve as acceptor molecules for sperm-associated acrosin inhibitor (SAAI). The attachment of SAAI to the surface molecules of the sperm can stabilize ZP-binding sites and protect the sperm against proteolytic degradation (2).

Heparin-binding proteins (esp. DQH, AQN, AWN) and their aggregated forms (fractions II and III) showed affinity to yeast mannan and the oviductal epithelium cells and fluid. Indirect immunofluorescence (IMF) showed that AQN 1 spermadhesin and fraction II bind to apical glycocalyx of the ampulla, the isthmic and uterine tubal junction regions of the oviductal sections. IMF proved the recognition of AQN 1 and fraction II and mannosyl components of oviductal epithelium. We suggest that these proteins on the sperm could enable the sperm binding to oviductal epithelium and thus participate in the formation of the sperm oviductal reservoir. Moreover, the ability of hyaluronic acid to inhibit the interaction of sperm surface proteins to the oviduct might play a role in the sperm release from the oviductal reservoir and in the capacitation process (3, 5).

We have found human seminal plasma proteins immunobiochemically related to boar AQN and AWN spermadhesins. We characterized the AQN-related proteins (hSA = human spermadhesin-like proteins). On Western blot, we immunodetected 14, 16, and 18 kDa hSA proteins cross-reacting with rabbit AQN antibodies. The similarity of biochemical properties of hSA and AQN proteins (relative molecular masses, pI, existence of non- and N-glycosylated forms, N-terminal amino acid sequence identity) imply that hSA proteins are structurally related to boar AQN spermadhesins. However, localization of hSA proteins on the sperm tail and neck suggests that their biological role differs from that of boar AQN spermadhesins located on the sperm head (4).

During pregnancy in mice, thymus weight is lowered, and leptin concentration in blood plasma and in adipose tissue is enhanced in the latter half of pregnancy. Immunosuppressive fraction (ISF) of boar seminal vesicle fluid (ISF), the heterodimer of PSP I and PSP II proteins, administered at the beginning of pregnancy, significantly lowered the leptin concentration both in blood plasma and adipose tissue of pregnant mice. Besides, ISF treatment compensated the loss of thymus mass but did not affect pregnancy and litter size (6).

Cardiovascular effects of LVV-haemorphin-7, a fragment of the -chain of haemoglobin, were studied in conscious spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats by radiotelemetry. Intraperitoneal injection of haemorphin in a dose of 100 g/kg significantly decreased blood pressure in SHR, whereas a negligible effect was seen in normotensive WKY rats. The blood pressure decrease was accompanied by reduction of heart rate. Metabolites of haemoglobin can be biologically active in blood pressure regulation especially in hypertensive rats, but the precise mechanism should be elucidated (7).

The work of the group was supported by the Grant Agency of the Czech Republic (grants Nos. 303/02/0433, 303/02/P069, 303/04/P070, 303/05/0614), and by the Ministry of Health of the Czech Republic (grant No. NJ/7463-3).

Publications

1. Jonáková, V., Tichá, M.: Boar seminal plasma proteins and their binding properties. *Coll. Czech Chem. Commun. 69: 461-475, 2004.*

2. Jonáková, V., Jelínková-Slavíčková, P.: Serine proteinases and their inhibitors in fertilization. *Biomarkers and Environment 8: 108-110, 2004.*

3. Jelínková, P., Liberda, J., Maňásková, P., Ryšlavá, H., Jonáková, V., Tichá, M.: Mannan-binding proteins from boar seminal plasma. *J. Reprod. Immunol. 62: 167-182, 2004.*

4. Kraus, M., Tichá, M., Železná, B., Pěknicová, J., Jonáková, V.: Characterization of human seminal plasma proteins homologous to boar AQN spermadhesins. *J. Reprod. Immunol. 65: 33-46, 2005.*

5. Liberda, J., Maňásková, P., Prelovská, L., Tichá, M., Jonáková, V.: Saccharide-mediated interactions of boar sperm surface proteins with components of the porcine oviduct. *J. Reprod. Immunol., in press.*

6. Veselský, L., Holáň, V., Dostál, J., Železná, B.: Boar seminal immunosuppressive fraction attenuates the leptin concentration and restores the thymus mass during pregnancy in mice. *Reproduction 127: 581-585, 2004.*

7. Čejka, J., Železná B., Velek J, Zicha J, Kuneš J.: LVV-hemorphin-7 lowers blood pressure in spontaneously hypertensive rats: radiotelemetry study. *Physiol. Res. 53: 603-607, 2004.*

Animal Facilities

Experimental Animal Farm – Koleč

Head: Miloslava Vilhelmová

Technicians: Alena Eisensteinová, Eva Fišerová (part time), Zdena Koptová, Alena Porazilová, Radmila Skoková, Jaroslava Strnadelová, Jiřina Urbánková, Milena Vaverková, Jaroslava Vlasáková

The facility is responsible for the breeding and improvement of the model system of poultry, which consists of inbred and congenic lines of chickens, outbred lines of chickens and an inbred line of ducks. The main part of this model system is a unique group of congenic and recombinant-congenic lines of chickens based on inbred line C. The complete list of available lines of chickens with the essential data about their history and genetics is given in the Table.

Line	Breed	Origin	Inbreeding	Colour	MHC	Sensitivity to ALV	ALV endogenes (expression)
INBRED LINES							
Congenic lines based on the C line							
CB	White Leghorn	RPBS 1932, Prague 1958	F25 + 45	I (white)	B^{12}	C/AE	gs^- chf$^-$ *ev-17*$^+$
CC		Prague 1963	F25+6+39	I (white)	B^{4}		*ev-17*$^-$
CB.7		Prague 1967	N9 F7 N4 F16	I (white)	B^{7}		
CC.13		Prague 1983	N9 F11	I (white)	B^{13}		
CB.9		Prague 1985	N9 F9	I (white)	B^{9}		
CB.15		Prague 1985	N9 F9	I (white)	B^{15}		
CB.R1		Prague 1975	N3 F25	I (white)	$B^{12r1(F12G4)}$		
CC.R1		Prague 1977	N6 F20	I (white)	$B^{4r1(F4G12)}$		
CC.R2		Prague 1978	N4 F22	I (white)	$B^{4r2(F4G7)}$		
CB.R4		Prague 1986	N9 F8	I (white)	$B^{21r3(F21G15)}$		
CC.R4		Prague 1986	N5 +N4 F7	i,E,B (barred)	$B^{21r3(F21G15)}$		
CB.R5		Prague 1989	N10 F4	I (white)	$B^{12}/B^{15r1(F15G21}$		
CC.21		Prague 1986	N9 F8	i,E (black)	B^{21x}		
CC.21-I		Prague 1997	N2 F5	I (white)	B^{21x}		
CC.ev^{17+}		Prague 1993	N2 F7	I (white)	B^{4}		*ev-17*$^+$
CB.ev^{17-}		Prague 1993	N2 F7	I (white)	B^{12}		*ev-17*$^-$

Other inbred lines

WA	Barred Leghorn	RPBS 1941, Prague 1962	?+F19+19G20	i,E,B (barred)	B^{9}	C/BE	gs^{+}
M	Black Minorca	Prague 1956	F48	i,E (black)	B^{21x}		
BLi	Brown Leghorn	Prague 1966	F_x=0.78+F25	e^{+} (wild)	B^{129}	C/E	gs^{+}
L 15B	White Leghorn	RPRL 1939, Prague 1977	F_x=0.95+F18 G4 F23	I (white)	B^{15}	C/C	gs^{-}
H6	White Leghorn	RPRL, HPRS, Prague 1989	F_x=0.99+F14	I (white)	B^{2}	C/E	

OUTBRED LINES

BL	Brown Leghorn	HPRS, Prague 1966	Avoidance of inbreeding	e^{+} (wild)		C/E	gs^{-}
P	Brown Leghorn	AUOC, Prague 1986	Avoidance of inbreeding	e^{+} (wild)			Free of endogenous DNA sequences of ALV
S	Barred Leghorn	Prague 1981	Avoidance of inbreeding	e^{+},sg,B (barred)	¨		

Laboratory Animal Facility – Krč

Head: Michael Boubelík

Deputy: Zuzana Žižková (since May 2005)

Technicians: Světlana Bonová, Blanka Kotková (since December 2005), Daniela Kratochvílová (until December 2005), Miloslava Kudličová (since September 2005), Pavel Lacina, Michaela Lišáková, Veronika Lorincová, Pavla Sojáková (until April 2005), Kateřina Ševčíková, Ludmila Šimečková, Markéta Tyšerová, Hana Vaňková

The facility is responsible for the health and welfare of laboratory animals and for improvement of the stock. It offers information, advice and assistance to experimenters during their work with laboratory animals. According to the law on protection of the laboratory animals against cruelty, the facility is responsible for routine examination of animals and monitoring of their health and genetic status. The facility is divided into an SPF unit and a monitored unit. Since 2004 a third unit – SPF unit for genetically monitored animals (GMO) – works as a new part of the facility.

Mice required for grants are produced under SPF conditions. Production concentrates mainly

onto gene knock-out and transgenic animals, mice from important genetic recombinant lines and mutants and consomic strains. Until now more than 50 such strains have been kept. The SPF unit has a section of isolators, which serves for introduction of new strains into the SPF unit. All animals are derived by hysterectomy and reared by foster mothers. Before introduction into the SPF unit the mice undergo complete bacterial, viral and parasitological examinations. All animals maintained in the SPF unit are examined for their health status according to FELASA recommendations. Strains are monitored genetically, each strain every two years. Material outgoing from the SPF unit is also frequently monitored. Bedding, water and cages are sterilized by autoclaving, the diet is irradiated. The personnel are only allowed to work under strict SPF condition. The GMO unit is open for other Institutes of the Academy of Sciences involved in biomedical research.

The monitored unit serves for routine short-time experiments. Animals in this unit undergo routine examination for their health status. The unit includes a small laboratory designed for conducting experiments on mice to avoid their possible stress during experimental procedures. Mice used in experiments are supplied from our own SPF unit or from commercial breeders who are licensed for production of the animals.

All researchers allowed to work in the monitored unit, as well in both SPF units, are trained and licensed for work with laboratory animals. Also the staff members of the facility are trained and licensed to carry out breeding and experimental work on laboratory animals according to the law for animal protection against cruelty, which is fully compatible with the pertaining EU directive.