Academy of Sciences of the Czech Republic
Institute of Molecular Genetics

Scientific Report 2002 – 2003

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Director's Introduction

When I became Director of the Institute four years ago, I had a reasonably good concept of how the Institute should develop and promote current trends in molecular genetics at the Academy of Sciences and how to disseminate these new trends to other institutions and laboratories in the Czech Republic. Now, after four years, I am gratified to say that several of my goals have been realized but that a few more challenges remain.

A major achievement is developing new research programmes at the Institute, such as molecular developmental genetics, and also a closer collaboration of individuals and entire groups within and outside the Institute. This has been facilitated by the establishment of Research Centers, two of which are coordinated by the Institute. The *Center for Integrated Genomics* consists of three groups from within the Institute, and additional groups from Charles University, Institute of Physiology of the Academy, and the Center for Molecular Genetics of the Institute of Chemical Technology. Altogether this Center comprises 49 scientists, technicians and support personnel. The *Center for Molecular and Cellular Immunology* consists of two groups from the Institute and another group at Charles University. The Center staff includes 47 members. Additional 27 persons are involved in the *Center for Molecular Biotechnology* coordinated by the Academy's Institute of Microbiology.

Currently, 22 groups at the Institute are engaged in research in the fields of immunology, microbial and mammalian genomics and bioinformatics, gene expression, oncogenes, retrovirology, cytoskeleton structure, molecular mechanisms of fertilization, cell signalling, molecular developmental biology and structural biology. To keep abreast of the latest developments in science, we have established new groups usually led by young colleagues, in many cases those returning from extended stays abroad at leading laboratories. All major methods of molecular genetics and cell biology are now routinely used by the scientists and technicians of the Institute of Molecular Genetics.

Scientists at the Institute publish about 200 papers each year, most of them in recognized international journals. In addition, the Institute publishes two academic journals: *Folia Biologica (Praha)* for original papers in molecular and cell biology, and *Biologické Listy* for review articles in Czech on various aspects of contemporary biology. These review articles serve primarily students and with these the Institute is also able to assist universities in updating curricula. In addition, many of us are involved in teaching. Of special importance is the pilot Ph.D. course, *Advances in Molecular Biology and Genetics*, organized annually by the Institute and attended by graduate students from the entire country. The Institute also collaborated in offering a special EMBO course of molecular genetics for secondary school teachers. Fifty-one students are working on their Ph.D. theses in the Institute's laboratories and 18 students on their Master's theses.

The Institute represents the Czech Republic in several leading scientific bodies, including the *European Molecular Biology Conference* (EMBC), the *European Science Foundation* programme on *Integrated Approaches in Genomics*, and in the NATO Steering Group of the *Science for Peace* programme. Four Institute scientists have been elected members of EMBO. Others serve on 18 Editorial Boards of scientific journals. Every year we organize one or two international meetings on various

aspects of modern biology. For example, the first European conference of ESF on *Functional Genomics and Disease* was organized in 2003 with 600 participants. All Institute groups were successful in receiving grants, both domestic and international, including seven Howard Hughes Medical Institute grants and two Wellcome Trust grants. This indicates that the Institute enjoys a respectable international standing.

Recently, several young colleagues started new biotech companies. The Institute supports this trend by helping and collaborating with them.

We at the Institute of Molecular Genetics are well aware that it is the responsibility of scientists to keep the public informed about research being conducted in our laboratories. To this end we work together with the media in explaining the importance of science in general and molecular genetics in particular. The Institute of Molecular Genetics is always open to all interested persons.

Václav Pačes

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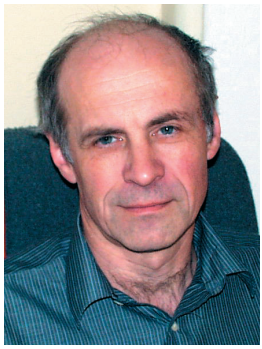

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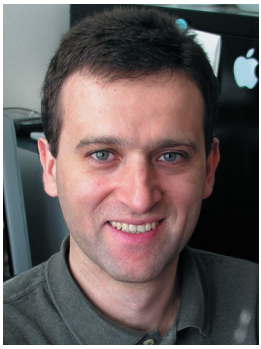

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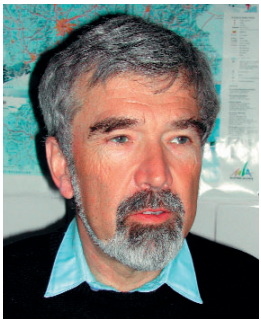

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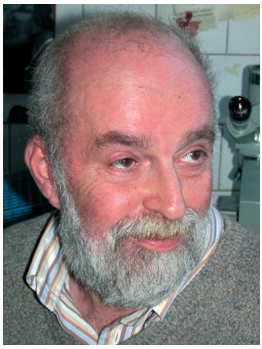

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Secretary: Eva Tvrzníková (part time)

Introduction

A major topic of our laboratory has been for many years the 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 membrane rafts, termed also GEMs (glycolipid-enriched membrane microdomains). These minute 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 GEMs (which were actually co-discovered by our laboratory more than ten years ago) we are interested in thorough functional characterization of their protein components, namely transmembrane adaptor proteins (LAT, PAG/Cbp, NTAL/LAB, LIME) and their involvement in immunoreceptor signalling. This work has been recently based mainly on a close collaboration with the laboratory of Prof. Burkhart Schraven (University of Magdeburg).

Results

The most important result of our laboratory in 2002-2003 was identification, structural and functional characterization of two novel transmembrane adaptor proteins named by us NTAL (Non-T cell activation linker) (1) and LIME (22). These palmitoylated proteins are localized in GEMs (membrane rafts). NTAL (also known as LAB) is structurally and evolutionarily related to LAT, a key adaptor protein of T lymphocytes. NTAL is expressed in B lymphocytes, NK cells, monocytes and mast cells but not in resting T lymphocytes. NTAL is the product of a previously identified *WBSCR5* gene of so far unknown function. NTAL becomes rapidly tyrosine-phosphorylated upon cross-linking of the B-cell receptor (BCR) or of high-affinity Fcγ- and Fcε-receptors of myeloid cells and then associates with cytoplasmic signalling molecules Grb2, Sos1, Gab1 and c-Cbl.

NTAL expressed in a LAT-deficient T-cell line, J.CaM2.5, becomes tyrosine-phosphorylated and rescues activation of Erk1/2 and minimal transient elevation of the cytoplasmic calcium level upon TCR/CD3 cross-linking. Thus, NTAL appears to be a structural and possibly also functional homologue of LAT in non-T cells.

LIME (Lck interacting molecule) is expressed predominantly in T lymphocytes. Human LIME is a strongly basic polypeptide of 295 amino acid residues. In its cytoplasmic domain it contains five tyrosine residues, which become tyrosine phosphorylated after cross-linking of the CD4 or CD8 co-receptors. Phosphorylated LIME associates with the Src-family kinase Lck and its negative regulator, Csk. Ectopic expression of LIME in Jurkat T cells results in an increase of Csk in lipid rafts, increased phosphorylation of Lck and higher Ca^{2+} response to T-cell receptor complex stimulation. LIME thus appears to be involved in regulation of T-cell activation by co-receptors. A model is proposed how LIME may regulate the activity of the key kinase Lck.

As can be seen from the list of publications below, we also wrote several reviews and invited commentary articles and we participated in a number of collaborative studies concerning membrane rafts and their components, MHC proteins and other leukocyte membrane proteins.

During the last 15 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). These monoclonals are available to other researchers either directly on request or commercially through an Institute-linked company, Exbio (www.exbio.cz).

The work of the group was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (research grant A7052904), Ministry of Education, Youth and Sports of the Czech Republic (project Center of Molecular and Cellular Immunology (LN00A0026)), international collaboration project EUREKA No. 2334 COCANAL) and The Wellcome Trust (grant No. J1116W24Z).

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Introduction

The research programme of the laboratory is focused on the mapping and functional analysis of genes that control the immune response and resistance to infection. 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 27 novel loci that control the immune response and resistance to infection.

Results

Genetic Dissection of Complex Disease: *Leishmania major* Infection Model

The human genome project has awakened interest in the genes that influence common multigenically controlled diseases, as their effects are likely responsible for a large part of human morbidity and mortality. The identification of these genes could help to assess the individual propensity to specific pathogenetic pathways, the prognosis of a disease, and to optimize the therapy, thus bringing closer the ideal of personalized medicine. 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*, which infect mononuclear phagocytes of vertebrate hosts. 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 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 the 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 (reviewed in ref. 6).

We have shown that the susceptibility to *L. major* is multigenically controlled (*Science* 274: 1392, 1996) and performed a systematic assessment of the role of host genes in clinico-pathological and immunological manifestations of *L. major*-induced disease using 20 RC strains derived from the susceptible strain BALB/c and the resistant strain STS. Disease or healing in different RC strains occurred in association with different components of the immune response. Moreover, some parameters of the immune response were highly correlated in some strains, but not at all in others. This shows that several patterns of the immune response may be associated with the same clinical outcome, depending on the host genotype (1).

Different Genetic Control of Cutaneous and Visceral Disease after *Leishmania major* Infection in Mouse

We have analysed the response of RC strain CcS-16, which is even more susceptible to *L. major* than BALB/c. In the (CcS-16 x BALB/c)F_2 hybrids we mapped three novel *Lmr* (*Leishmania major* response) loci that influence cutaneous or visceral pathology. *Lmr14* (chromosome 2) controls splenomegaly and hepatomegaly. On the other hand, *Lmr15* (chromosome 11) determines hepatomegaly only, and *Lmr13* (chromosome 18) skin lesions only. This data confirms the complex control of *L. major*-induced pathology, where cutaneous and visceral pathology are controlled by different combinations of genes. It indicates organ-specific control of anti-parasite responses. The definition of genes controlling these responses will permit a better understanding of pathways and genetic diversity underlying the different disease phenotypes (3).

Predictability of Phenotype

As our studies suggested the involvement of several genes in the control of each of the pathological and immunological parameters (*Genes Immun*. 1: 200, 2000 and refs. 2, 3), we tried to correlate the predicted effect of STS alleles of such loci present in the individual CcS strains with the actual phenotypes of these strains. This has been possible for the IgE levels, as this trait has been analysed in three strains CcS-5, -16 and -20 that cover together about 33% of the STS genome. As we detected in these strains nine *Lmr* loci influencing the IgE level, an extrapolation suggests about 30 *Lmr* loci polymorphic between BALB/c and STS that control this trait. For the seven IgE controlling loci that have a strong individual effect, we determined their genotypes in individual CcS strains and classified the impact of their alleles as increasing (+) or decreasing (-) the level of IgE. Subsequently, we summed the directional effects of these alleles for each CcS strain, which resulted in an "IgE grade" ranging from –3 to +1, and we compared the grade with the actual IgE levels in the individual strains (Fig. 1).

The general correlation of the presence of alleles increasing or decreasing the IgE with the actual IgE levels of the individual CcS strains

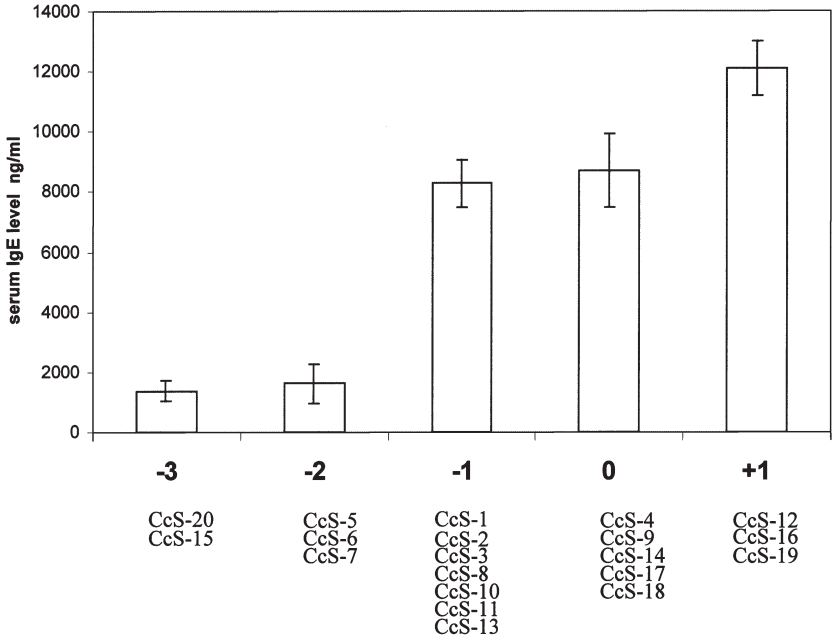

Fig. 1. Serum IgE level (mean ± SD) of the CcS/Dem strains with different "genotype IgE grade". Values of IgE levels of *L. major*-infected mice were taken from ref. (1). The actual and predicted phenotypes fit better than those reported in (1), as additional typing of DNA segments near *Lmr* loci in CcS/Dem strains led to their more precise mapping.

shows that when a sufficient number of loci is known, the prediction of phenotype is possible.

We also cooperated on cancer genetics studies (5).

Perspectives: The aim of our future studies is to map *Lmr* loci to 0.5-1 cM and to identify candidate genes by positional cloning. In parallel, we shall study the functional effects of these genes. We shall establish segments homologous to IgE controlling loci on human chromosomes and we will test whether these genes control the IgE level in Czech atopic families.

Cooperations: The RCS system and the support with genetic mapping is provided by Prof. Peter Demant (Roswell Park Cancer Institute, Buffalo, USA). In *L. major* infection studies we cooperate with Dr. M. Svobodová and Prof. P. Volf (Department of Parasitology of the Faculty of Science, Charles University, Prague) and Dr. E. Nohýnková (Department of Tropical Diseases, 1st Medical Faculty, Charles University, Prague). The role of neutrophils in the *L. major* infection is analysed in cooperation with Prof. George. A. DosReis (Federal University of Rio de Janeiro, Rio de Janeiro, Brazil).

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Publications

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Tumour Immunology

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Introduction

The long-term research programme of the laboratory is focused on investigation of the cell signals involved in the induction of tumour immunity, with the final aim to supply the cancer-bearing individuals with the missing signals by administration of recombinant cytokines, dendritic cells loaded with oncoproteins, or by gene therapy using vaccination with tumour cells carrying the inserted genes for the respective molecules.

Results

Various forms of the cytokine therapy based on utilization of recombinant interleukin 2 (IL-2), interleukin 12 (IL-12), interferon (IFN) alpha and gamma, and granulocyte-monocyte colony stimulating factor (GM-CSF) were investigated and compared. The gene therapy programme has used insertion of the genes coding for immunostimulatory molecules, such as IL-2, CD80, or GM-CSF into tumour cells, followed by utilization of the resulting, genetically modified irradiated tumour vaccines for treatment of cancer in preclinical tumour systems. The highlights for the research programme were studies of optimal combinations and timing of the activation and immunostimulatory signals for therapy.

Most of our experimental studies indicated that particularly non-generalized early forms of cancer, small primary tumours, minimal residual tumour disease induced by cytoreductive chemotherapy or surgery, and micrometastases should be considered for the immunotherapy of cancer.

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 designated as TC-1, MK16 were utilized. The murine HPV 16-associated carcinoma systems developed for the studies are mimicking human HPV16-associated carcinomas with regard to the aetiology, ability to metastasize and absence (MK16) or presence (TC-1) 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^- HPV16-associated tumours, comprising approximately 60% of human cervical carcinomas.

In this model system we have for the first time performed the gene therapy of MHC class I^- tumours and we have compared these results with the results of therapy of MHC class I^+ tumours of identical aetiology and haplotype. In clinically relevant models of residual tumour disease after tumour excision and chemotherapy we found that MHC class I^- as well as MHC class I^+ tumour residua can be successfully treated by administration of recombinant IL-2 or GM-CSF, or by administration of an irradiated, IL-2-producing tumour vaccine. A therapeutic effect of the irradiated vaccine producing GM-CSF was only found in the residual disease of MHC class I^+ tumours

after chemotherapy. The adjuvant effect of the administration of recombinant IL-12 after chemotherapy using ifosfamide derivative CBM-4A was compared in mice carrying MHC class I^+ and MHC class I^- tumours. It has been found that IL-12 substantially enhances the therapeutic effect of chemotherapy in MHC class I^+ tumours, while in MHC class I^- tumours this was only observed when the combination of cytokines, IL-12 and IL-2, was used.

The *in vivo* effects of bone marrow-derived dendritic cells (BMDC) were examined in mice carrying syngeneic, HPV16-associated MHC class I^- carcinoma transplants. It has been shown that local pre-treatment with BMDC inhibited growth of a subsequent challenge inoculum of this carcinoma. Similarly, treatment of mice carrying small HPV16-associated carcinomas and of those with surgical minimal residual tumour disease performed with BMDC substantially inhibited tumour growth. These findings suggest that local concentration of BMDC at the tumour site can influence the development of HPV16-associated, MHC class I^- tumours and that it is important for prognosis of the tumour bearer. Tumour lysate-loaded BMDC inhibited growth of the HPV16-associated carcinomas more vigorously than unloaded BMDC. *In vitro* priming studies of the antitumour responses in this experimental system revealed that the priming activity of HPV16 $E6/E7^+$ tumour lysate was substantially higher than that of the HPV16 $E7_{(49-57)}$ RAHYNIVTF immunodominant peptide.

A technically important finding was that the established lines of dendritic cells (DC 2.4, JAWS II) displaying similar properties as freshly prepared dendritic cells from bone marrow can be used for vaccination. This finding will substantially increase the reproducibility of the results obtained with the dendritic cell-based vaccines. The antitumour effect of unloaded dendritic cells prepared from the bone marrow of syngeneic mice was compared with the effect of established dendritic cell lines DC 2.4 and JAWS II using prophylactic administration in mice that were later inoculated with the tumour; it was found that the prophylactic administration of BMDC, DC 2.4 and JAWS II cells had a comparable inhibitory effect on tumour growth. Similarly, the therapeutic effect of BMDC, DC 2.4 and JAWS II cells administered at the site of the growing tumour, the antimetastatic effect and the effect on the reduction of recurrences after surgical removal of the tumour were also comparable. A prospective technique for preparation of vaccines from dendritic cells is *in vitro* hybridization of cell lines from dendritic and tumour cells. For these purposes we have prepared hybrid cell lines DC 2.4 x TC-1 and used these hybrid lines for therapeutic administration in the vicinity of the TC-1 tumours growing in syngeneic mice. We have compared the therapeutic effect of hybrid cells administered 24 hours after fusion with the effect of hybrid cells selected for 3 weeks in the HAT medium. It has been found that administration of both types of the dendritic cell-based vaccines significantly reduced the number of tumour-bearing mice as well as the size of the transplanted tumours.

Experiments were designed to examine whether local cytokine therapy of HPV16-associated tumours results in inhibition of their lung metastases. It has been found that therapy of small s.c. tumour transplants by peritumoural administration of recombinant IL-2 or IL-12 inhibited growth of the s.c. tumour inocula and reduced the number of their lung metastases. To investigate the antimetastatic effect of IL-2 and IL-12 in a clinically more relevant setting, surgical minimal residual tumour disease was developed by removal of subcutaneously growing tumours, 8-12 mm in diameter, and the operated mice were injected with IL-2 or IL-12 at the site of the operation. Treatment with IL-2 significantly reduced the percentage of local tumour recurrences as well as the number of lung metastases, whereas the effect of IL-12 was substantially weaker. It must be stressed that the antimetastatic effect of IL-2 was also expressed in the cases of a negligible

effect of IL-2 on the growth of the s.c. tumour and thus can be considered as a more sensitive indicator of the antitumour effect of the cytokine.

To assess whether the MHC class I expression by the MHC class I^- $HPV16^+$ tumour cells could be induced by procedures leading to IFNγ production, the $HPV16^+$ MHC class I^- tumour cells were grown *in vitro* in the medium supplemented with IFNγ and then re-examined in flow cytometry with FITC-anti-mouse $H\text{-}2K^b/H\text{-}2D^b$ monoclonal antibodies. It has been found that the expression of MHC class I molecules on HPV16-associated tumour cells could indeed be induced by the treatment with IFNγ.

We have investigated whether the experimental tumours induced by the HPV 16 virus that differ in the presence of the MHC class I molecules are capable of cross-reacting, i.e. whether immunization by the MHC class I^+ tumour elicits resistance against the MHC class I^- tumour and vice versa. It was found that these two types of tumours do not immunologically cross-react. The immunological non-cross-reactivity was found despite the presence of immunogenic oncoproteins E6/E7 HPV 16 and Ha-ras in both types of tumours and despite the finding that immunization by the MHC class I^+ tumour elicits resistance against the MHC class I^+ tumour, and immunization by the MHC class I^- tumour elicits resistance against the MHC class I^- tumour. These findings have a priority character and will have a key significance for further design of antitumour vaccines, if it is possible to confirm their general validity.

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Transplantation Immunology

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Introduction

Immunological rejection represents the major obstacle for further development of clinical transplantation**.** Therefore, recognition of the cellular and molecular mechanisms of immunological reaction and obtaining the ability 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 subopopulations, 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 used 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 the immunologically privileged site. Using *in vitro* models of transplantation reaction, such as mixed lymphocyte culture or cytotoxicity tests, the molecular mechanisms of immune reaction were tested. The research is 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 that 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 the results obtained, new immunoregulatory approaches are proposed and tested. The ultimate goal of the research is recognition of the mechanisms of specific immune response and a proposal and testing of novel approaches how to increase or suppress the immune response in a desirable manner.

Results

We demonstrated that nitric oxide (NO) produced by graft-infiltrating macrophages represents an important effector molecule contributing to the damage of the grafted tissue (1). The production of NO is gently regulated by T-cell subpopulations and various cytokines. In addition to its role as the effector cytotoxic molecule, NO acts as an immunomodulator. Using specific inhibitors of inducible NO synthase we demonstrated that NO selectively modulates production of Th1 and Th2 cytokines in activated T lymphocytes (2).

Specific features of immunity in the eye as in the model of an immunologically privileged site

were studied on the model of corneal transplantation. In clinic, corneal transplantation represents the most common and the most successful type of transplantation. However, a significant proportion of the grafts are lost due to the immunological rejection. Therefore, we established an experimental model of corneal transplantation and we used this model for studying the immune reaction after corneal allo- and xenotransplantation. We pointed out the importance of the surgery technique for the outcome of transplantation (3) and we described the distinct roles of individual draining lymph nodes in the rejection or tolerance of corneal allografts (4). Namely, the submandibular lymph node has been shown to be responsible for induction of rejection reaction, whereas its nearest neighbour, the superficial cervical lymph node, is involved in the induction of immune privilege. Our other studies demonstrated that the cornea is not only a passive barrier protecting the eye, but that corneal stroma cells produce a factor that selectively inhibits production of anti-inflammatory cytokines (14). The cornea can thus regulate the expression of protective inflammatory reactions in the environment of immune privilege in the anterior part of the eye. To mimic the clinical situation, where a very high proportion of corneal allografts in high-risk recipients is rejected in spite of intensive immunosuppressive treatment, we prepared mouse high-risk recipients. The recipients of corneal allografts had their graft bed prevascularized by sutures made two weeks before transplantation. Corneal allografts in such recipients were rejected promptly and the suppression with classical immunosuppressive drugs, such as cyclosporin A or mycophenolate mophetil, failed (13). However, treatment of the graft recipients with monoclonal antibody anti-CD4, but not anti-CD8, prevented graft rejection in the majority of recipients (13). These results suggest that the treatment with anti-CD4 antibody may be effective in corneal graft recipients where other immunosuppressive approaches failed.

Well-established methods for detection of cytokines and for evaluation of their immunoregulatory role enabled us to test the immunoregulatory effects of the boar seminal immunosuppressive fraction (5, 8), various psychostimulants (6, 12) and opiates in experimental models (7, 10) or in heroin addicts and patients maintained on methadone (11). In collaboration with French colleagues we participated in the discovery that DNA vaccination can induce a protective immune response against leukaemia progression in mice (9).

The work of the group was supported by grant No. 310/02/D162 from the Grant Agency of the Czech Republic, grants Nos. NI/6659-3, NF/6824-3 and NI/7531-3 from the Grant Agency of the Ministry of Health of the Czech Republic, project LN00A026 from the Ministry of Education, Youth and Sports of the Czech Republic and by Wellcome Trust grant No. 061367/Z/00/Z.

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Genomics and Bioinformatics

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Introduction

Genomics and bioinformatics belong to the most dynamically 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 when the complete nucleotide sequence of the bacteriophage PZA DNA was determined. Since then, our laboratory has been developing new approaches and strategies to the large-scale DNA sequencing and was involved in several international genome projects including the Pseudorabies virus and bovine herpesvirus genome projects, the *Saccharomyces cerevisiae* genome project and the *Rhodobacter capsulatus* genome project. Information generated in these projects was used in evolutionary studies and recently also in biotechnological applications. In collaboration with other Institute groups, individual members of the Department became involved in the characterization of several developmental genes and in studies on transcriptional profiles of expression of several mouse genes. With the advances in the human genome project and mouse genome project, we are currently analysing these genomes especially as they concern foreign elements present in these and other (e.g., bacterial) genomes.

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

Results

In collaboration with the laboratory of Zbyněk Kozmik, the group has characterized (1, 2) two developmental genes of different chordate organisms. We found mammalian orthologues of *osa*, a gene of *Drosophila melanogaster,* which encodes a nuclear protein. The mouse orhologue *Osa1* is expressed during development. The cDNA nucleotide sequences derived from mouse and human genes revealed three developmentally conserved domains (1).

The structure has been described for the amphioxus *AmphiVent* gene, a homologue of vertebrate *Vent* genes. We found that *AmphiVent* encodes a protein distinguished by an unusual homeodomain in which the forty-seventh amino acid is a threonine instead of the canonical isoleucine. Only the mammalian Vent and ladybird-like proteins are similar enough in the flanking regions of the homeodomain. This suggests that in spite of their evolutionary distance, these genes are close relatives with analogous functions. The phylogenetic tree confirmed this suggestion (2).

The international genome project of *Rhodobacter capsulatus* has been completed (3). Several genes with possible biotechnology applications are now being characterized (4).

Special attention was given to human genome analysis, namely to the isochore structures of individual human chromosomes (5-7). In addition, distribution of Alu and LINE repetitive

sequences was characterized (8). HERVd, the database of human endogenous retroviruses, was assembled (9,10). 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.

In collaboration with botanists, phylogenetic analyses based on chloroplast, rRNA, and nuclear DNA sequences were performed for several plant types (11-14).

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Mammalian Molecular Genetics

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Introduction

Genomes of most classical laboratory mouse inbred strains originated predominantly from the *Mus musculus domesticus* subspecies and in less than 20% from the closely 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 variants in their phenomes. Thus, genetic crosses utilizing classical laboratory strains and strains derived purely from the *M. m. musculus* subspecies are likely to be highly informative for functional genomics studies.

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 are mapping and positional cloning of hybrid sterility genes, and functional genomics using the PWD strain and chromosome substitution strains of mice as models. Finally, the phenome analysis and expression profiling of mice with segmental trisomy Ts43H is being set up as a mouse model of human aneuploidy syndromes, such as M. Down syndrome.

Results

The project on generating 21 inter-subspecies mouse chromosome substitution strains was on target for the third year. As of December 2003, six consomic strains were finished and 15 strains have reached the last generation of backcross breeding (N11). The conplastic strain, carrying PWD mitochondrion on the genetic background of the B6 strain, was at N8. Altogether 315 SSLP markers have been used during the project and over 40 000 genotypes have been analysed so far. To enhance the utility of chromosome substitution strains, the genomic BAC library from the PWD/Ph male mice was constructed with 100 000 clones (average size > 100 kb) (Gregorová, Štorchová, Jansa, Divina, Landíková and Forejt, in progress).

Segmental trisomy of the proximal part of Chr 17, Ts43H, has been characterized as a mouse model of human aneuploidy syndromes (4). In the first study on gene expression within the trisomic segment, the maternal expression of the imprinted *Igf2r* gene was observed regardless whether the trisomic animal carried two maternal or two paternal copies of the gene. The quantitative, real-time RT PCR showed the same level of total

Igf2r mRNA in maternal as well as in paternal trisomics, suggesting an unknown gene-dosage control mechanism of this imprinted gene (1).

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 seems to be identical with *Hst1* (5).

The synteny conservation of members of eukaryotic operons was investigated by mapping their orthologues in other eukaryotes. Although most of the orthologues are not syntenic, a few new examples of highly conserved syntenies were revealed (6).

The Mouse SAGE Site database of public available SAGE libraries generated from mouse tissues and cell lines was constructed (3). Currently, the database contains data from 65 mouse SAGE libraries and provides easy-to-use tools for browsing, comparing and searching the SAGE data. The Mouse SAGE Site is freely accessible on the website as follows: http://mouse.biomed.cas.cz/sage/.

Our group also participated as collaborator in another project on mammalian functional genomics, the rat model of human metabolic syndrome (2).

The work of the group was supported by the Grant Agency of the Czech Republic (research grant No 204/98/K015 and 204/01/0997) by the Czech Ministry of Education, Youth and Sports No. LN00A079 (Center of Integrated Genomics), by EU grant QLRT-2000-00233 and by the Howard Hughes Medical Institute.

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Cellular and Viral Genetics

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Introduction

The long-term interests of our group have been the interactions between invading retroviruses and the defence machinery of infected host cells. Heterotransmission of retroviruses, i.e. transfer from their natural host into the foreign species, has been a potent tool for such studies thanks to a broad scale of suppressive effects raised by non-permissive host cells against the non-adapted virus. The barriers against retroviral heterotransmission as well as the mechanisms by which retroviruses can overcome them and adapt to the new host are not well understood yet. The worldwide human immunodeficiency virus type 1 (HIV-1) epidemic is a clear example of previous heterotransmission from non-human primates to human. In addition to the experimental approach, a large amount of knowledge of virus-host coevolution comes from whole-genome studies of human endogenous retroviruses (HERVs) and retrotransposons.

An extreme example of interclass heterotransmission of Rous sarcoma virus (RSV) from chicken into non-permissive mammalian cells has been a key model for the postulation and evidence of the proviral state and oncogene transduction (17,18). Furthermore, RSV displays a handful of striking phenomena in the mammalian host cell, especially efficient transcriptional suppression of integrated RSV proviruses by *de novo* CpG methylation (17). Therefore, our experimental system is also interesting from the point of view of epigenetic silencing of integrated retrotransposable elements. Escape from this transcriptional control might, on the other hand, be useful in improving the long-term transcription of retrovirus-based vectors used for the gene transfer. We, therefore, look for the pattern of DNA methylation within proviral long terminal repeats (LTRs), leader sequences and cellular flanking regions, and we try to protect RSV proviruses using antimethylation signals. We suggest that the DNA methylation may be part of the natural barriers against retroviral transmission between heterologous host species.

We are also interested in the pathogenic consequences of persistent infection of avian leukaemia viruses (ALVs), because there are few

data for comparison between oncoviral and lentiviral pathogenesis (17). Previously, we have established our inbred flock of domestic ducks as a valuable model for studies on ALV pathogenesis in a semi-permissive host. Recently, we have started a new project of identification of the ASLV-C retrovirus receptor. For the aforementioned projects, 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) (9, 22).

Whole human genome surveys of HERVs, Alus and long interspersed nuclear elements (LINEs) have been proven as efficient tools in testing some of hypotheses raised by our previous findings on retroviral integration specificity and the effect of integration sites on retrovirus expression. Especially, the analysis of HERV-Ws turned to be fruitful in the current search for the role of HERV-Ws in human development.

The last but not least research topic in our group is the characterization of the novel tumour-associated CA IX protein with carbonic anhydrase activity. This is a cell surface protein expressed in a high percentage of certain human carcinomas (renal, cervical, lung, colorectal, etc.) displaying a cell-to-cell adhesive function. Our interest in CA IX is reasoned by the expectation that it can serve both as a diagnostic marker and a target molecule for therapy.

Results

We have shown previously that the RSV LTR is sensitive, at least in mammalian cells, to the transcriptional repression by DNA methylation. Along the same line, junction of a CpG island with RSV LTR resulted in partial resistance to the post-integrative inactivation and anti-methylation protection of the proviral reporter in mammalian cells.

To understand in more detail the mechanisms of *de novo* DNA methylation and transcriptional suppression of integrated retroviruses, we started to study **CpG methylation patterns in proviral LTRs** by the bisulphite technique. Two interesting observations resulted from this work: first, after exogenous infection of chicken cells, RSV proviruses lack almost any methylation, whereas in mammalian cells they are heavily methylated and suppressed (manuscript in preparation). This corresponds with a low level of methylation observed within HIV-1 LTR in *in vitro* infected cells (11) or AIDS patients. Second, in one interesting case of the simplified LTR, v-*src*, LTR provirus integrated into a hypermethylated genomic region, the provirus overcomes the suppressive constraints of the integration site, keeps its non-methylated status and demethylates the flanking sequences (12). This is the first example of such target site demethylation by retroviral integration. In addition to the exogenous retroviruses, we are also interested in CpG methylation of HERV-Ws, especially the syncytin, a captive HERV whose functional *env* is used in the process of syncytiotrophoblast differentiation.

Description of **the pathogenic effects of ALV in a semi-permissive foreign species host** went on with the characterization of wasting disease in ALV-C-infected inbred ducks. Clear symptoms of wasting appeared soon after hatching: decreased body weight, thymus involution, atrophy of bursa Fabricii, anaemia, and decrease in the antibody response (19). The pronounced wasting disease correlates with high mortality within three weeks after hatching; the remaining animals suffering a mild wasting recovered and represent the carriers of persistent virus infection.

We have employed the growing capacity of chicken genomic tools to the precise genetic **mapping of genes encoding receptors for ALV-A and ALV-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 and narowed the region to the BAC-mediated complementation screening in effort to identify the unknown subgroup C receptor (24).

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-resistatnt 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 are demonstrated (manuscript in preparation).

Current progress in the analysis of the human genome enables genome-wide screens and classification of endogenous retroviruses. We have initiated creation of an HERV database (http://herv.img.cas.cz) (3, 23) and further focused on the class W of HERVs. We have determined the genetic structure of all **HERV-Ws** found in the GoldenPath assembly of 87% of the human genome and described the presence of **numerous poly(A)-containing processed pseudogenes** truncated at the 5' end derived from this family. Our preliminary search for such pseudogenes in other HERV families shows that this frequency is unique for HERV-Ws. We have analysed the insertion sites of HERV-W processed pseudogenes and shown a strong preference for the insertion motif of long interspersed nuclear element (LINE) retrotransposons. Along the same line, the genomic distribution, evolutionary stability, and frequent truncations at the 5' end resemble those of the LINE-generated pseudogenes. We suggest that HERV-W processed pseudogenes arose by multiple and independent LINE-mediated retrotransposition of retroviral mRNA (1). The current search for HERV-Ws associated with several human disorders should concentrate on a small subset of transcriptionally competent elements omitting the high proportion of non-transcribed promoter-less pseudogenes.

LINEs and HERVs together make up some 25% of the human genome. They are known to share the same retrotransposition mechanism, the target-primed reverse transcription (TPRT). During this TPRT, however, the LINE-encoded reverse transcriptase often abortively dissociates from the RNA template, leaving a prematurely terminated, 5' truncated copy. We have analysed the **length distributions of LINEs and processed pseudogenes derived from HERV-Ws**. As expected, we have found bimodal length distribution with a majority of 5' truncated members terminated close to the 3' end and with a peak of complete full-length elements far above the previous estimates (2, Fig. 1). The characteristic, power-law length distribution indicates two important conclusions: (i) dissociation of reverse transcriptase from the template cannot be fully explained by low processivity modelled as a stochastic, Poisson-type process. (ii) Currently cited numbers of pseudogenes in the human genome are underestimated, since a large percentage of them are terminated in the 3' untranslated region and remain undetectable in translated homology searches of protein databases against the human genome.

The genome-wide approach was also used for the **analysis of integration sites of HIV-1 proviruses**. The public database by Carteau et al.

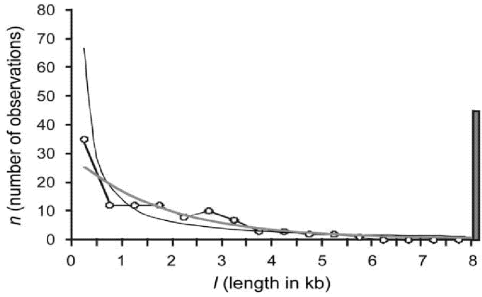

Fig. 1. Length distribution of HERV-W processed pseudogenes. Absolute numbers of HERV-W processed pseudogenes within 500 bp intervals are depicted in the plot. Power-law (black) and exponential (gray) fit functions are shown.

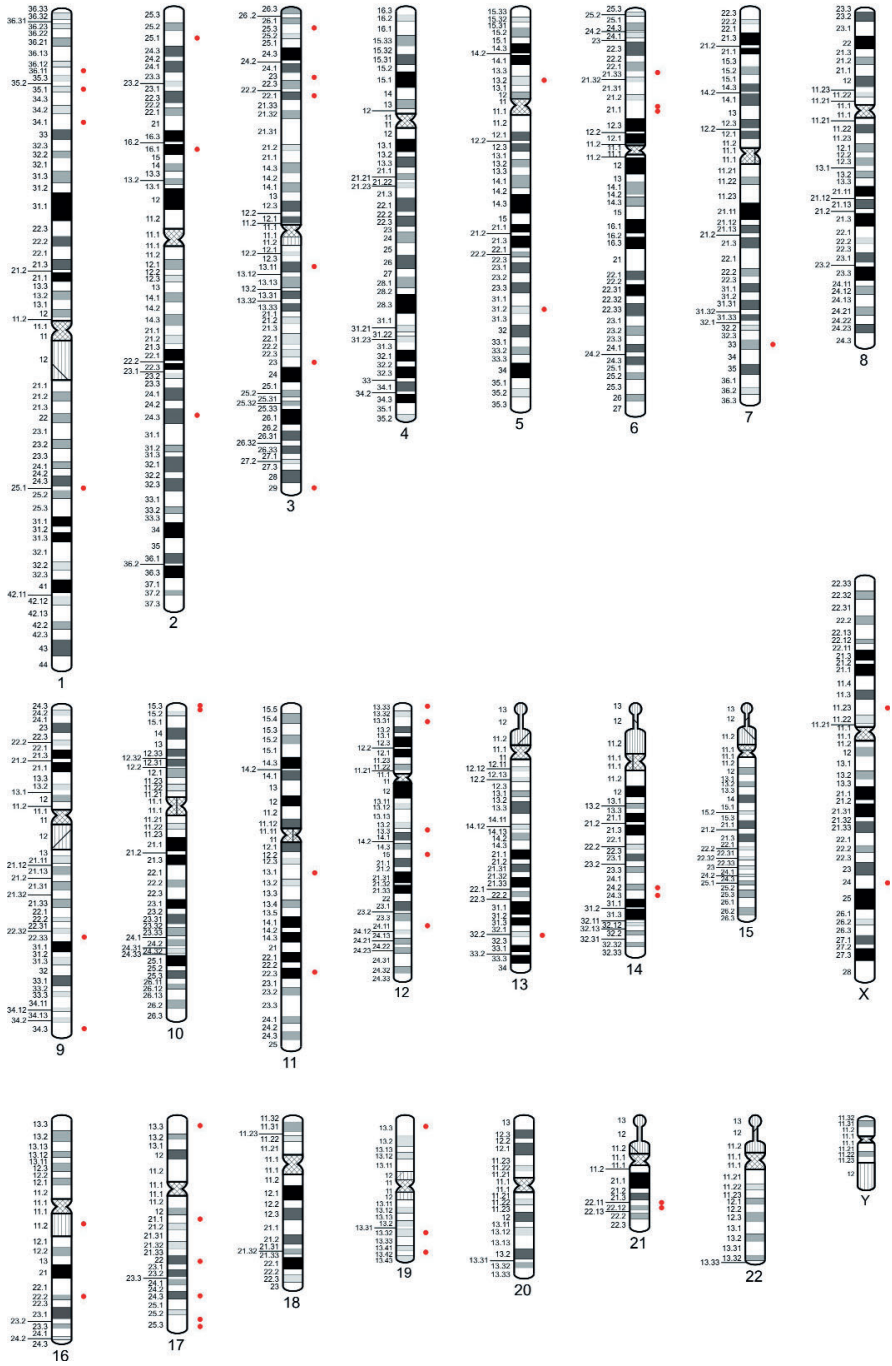

Fig. 2. Chromosomal positions of mapped HIV-1 integration sites (red dots).

(J. Virol. 72, pp 4005-4014, 1998) offered a set of 61 integration junctions isolated immediately after experimental infection of human T cells. We have assigned genomic positions of these short sequence tags using the GoldenPath assembly. Seventy-four percent and 85% of localized proviruses fall into known genes and in cytogenetic R bands, respectively (Fig. 2). We have also observed a strong integration bias for GC-rich DNA regions. These findings support the hypothesis of preferential integration of retroviruses into transcriptionally active and easily accessible decondensed chromatin regions (4).

To discern the biological role of CA IX, we have initiated an international project of **gene targeting *Car9***, the mouse homologue of the CA IX. The mice with null mutation of the *Car*9 were obtained by targeted gene disruption and their phenotype was described using biochemical and histochemical techniques. *Car9*-/- mice develop severe gastric hyperplasia of the glandular epithelium during the terminal gastric morphogenesis. This phenomenon is fully prominent in four-week-old animals. This hyperplasia is caused by increased cell proliferation detected by PCNA. Overproduction and aberrant distribution of mucus-secreting cells as well as depletion of pepsinogen-positive cells were observed. However, the elimination of CA IX does not lead to any gross physiological defect and blood pH, content of plasma electrolytes, and the serum level of gastrin are comparable with age-matched controls. The CA activity of CA IX is probably complemented by other enzymes of this family, whereas its role in the morphogenesis of stomach mucosa is non-redundant (10).

Following the potential **diagnostic and therapeutic use of CA IX**, we described that it has two major forms: (i) a cell-associated, transmembrane 54/58 kDa protein, expressed in gastric mucosa and in several types of cancer, and (ii) a soluble protein s-CA IX of 50/54 kDa, which is released into the culture medium or into the body fluids, most likely by the proteolytic cleavage of the extracellular part from transmembrane and intracellular sequences. Despite the fact that the level of this antigen in blood serum and urine of renal clear cell carcinoma (RCC) patients is about 1000 x lower than in medium of CA IX-positive tumour cell lines, there is a profound difference between RCC patients and control individuals. After nephrectomy, s-CA IX is cleared from the blood within a few days (15). Another approach might be the whole body macroautoradigraphy using the ^{125}I-labelled monoclonal antibody M75 specific for CA IX. Biodistribution and pharmacokinetics of intravenously administered ^{125}I-M75 was studied in nude mice xenografted with HT-29 human colorectal carcinoma (14).

The members of the Cellular and Viral Genetics group also participated as collaborators in other projects on the human genome isochore composition (5, 6, 7, 13), the role of CCR2 chemokine receptor and CCR2^{+} T cells during the inflamatory response in mice (21), the creation of a chicken DT40 bursal cell EST database (8), and the peptide-elicited immunoprotection against v-*src*-induced sarcomas in chicken (20).

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 301/99/0356, 524/99/0366, 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.

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Molecular Virology

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

The research efforts of the group focus on three 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 and of the nephrogenic blastema. For most experiments we use avian cells and tissues. In studies on cell fate determination, differentiation and malignant transformation of haematopoietic cells we use c-*myb* and v-*myb* genes and their specific mutants as tools to modulate developmental processes in these cells because Myb proteins are important regulators in the mentioned cell types. In studies on the nephrogenic blastema transformation we use the model of the chicken nephroblastoma induced by the MAV2 retrovirus.

Results

We have observed 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). In addition, v-Myb-bFGF cooperation results in a high proliferation rate and prolonged survival of erythroid cells in the culture. We also reported that c-myb downregulation during the terminal differentiation of erythroid cells is likely to be caused by the GATA-1 transcription factor (3). This work has been done in cooperation with Assoc. Prof. M. Zenke, Max-Delbrück Institute, Berlin.

We have recently observed that Myb proteins regulate several biological processes including cell fate determination, motility and survival in multipotent cells of the neural crest. 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 one of crucial myogenic factors. This interaction appears to be part of the differentiation programme in skeletal muscle formation.

Our studies on the mechanisms of malignant transformation of myeloid cells by the v-*myb* oncogene revealed an important protein-protein interaction through which the v-Myb oncoprotein activates regulators belonging to the Egr family. This interaction is very likely responsible for the uncontrolled proliferation of v-Myb-transformed leukaemic myeloid cells. The part of our work on the malignant transformation of haematopoietic cells has been focused on the development of porphyrin-based specific chemicals that could mediate the transport of antisense oligonucleotides into leukaemic cells (4). In addition, we attempt to optimize similar compounds linked to oligonucleotides for use as specific chemical nucleases. We also work on the development and application of porphyrin-based photosensitizers that upon photoactivation induce apoptosis in cells into which they were introduced. One such compound efficiently eliminates experimental tumours in mice. This work has been done in cooperation with Prof. V. Král, Institute of Technology, Prague. In cooperation with Dr. J. Schwarz, Institute of Haematology and Blood Transfusion, Prague, we participate in analyses of Flt3 receptor mutants in human myeloid leukaemia. Our work on nephrogenic blastema transformation induced *in vivo* by the integration of the MAV2 retrovirus 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). This conclusion has been supported by our recent work in which also other genes relevant for nephroblastoma initiation and progression have been identified.

The work of the group was supported by the Grant Agency of the Czech Republic (grants Nos. 301/98/K042, 204/00/0554, 301/01/0976, 304/03/0463, 203/02/0420 and 204/03/H066), and by the Grant Agency of the Academy of Sciences of the Czech Republic (research grant A5052309).

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Transcriptional Regulation

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Introduction

Our interest is focused on transcriptional regulation and its role in development. 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

We have shown previously that Pax6 overexpression in transgenic mice leads to development of lens cataract. We found previously that elevated levels of the Pax6(5a) splice variant lead to upregulation of alpha(5)beta(1) expression. We have now shown that Pax6 is also involved in the regulation of gamma-crystallin genes in the mouse lens (1).

We are using Amphioxus (*Branchiostoma floridae*), the closest living invertebrate relative of vertebrates, to address questions about the evolution of the animal body plan, with a special focus on sensory systems. We have therefore characterized the amphioxus orthologue of *Drosophila melanogaster* gene *dachshund*, which is a key gene regulating eye development (2). We have also contributed to the finding that Dachshund proteins interact with SMAD transcription factors, thereby inhibiting TGF-beta signalling (3).

We also focus on the regulatory mechanisms that control embryonic development of the mouse forebrain. In particular, we study the properties of neural stem cells and their genetic regulation. Using transgenic mice we inactivated the beta-catenin gene in the mouse cortex after embryonic day 11. We have shown that beta-catenin, as a key mediator of the Wnt signalling canonical pathway and component of the cytoskeleton, plays an important role in the proliferation of cortical and hippocampal progenitors. Further, beta-catenin is indispensable in the maintenance of multilayered architecture of the mouse cortex and in the migration of newborn neurons from the germinal zone to its final destination in the cortical plate (4).

The work of the group was supported by the Center of Integrated Genomics grant LN00A079.

Publications

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Molecular Genetics of Development

Head: Pavel Urbánek

Technician: Hana Ederová

Introduction

Our laboratory is interested in genes involved in normal development and physiology, as well as in various pathological conditions. Recently, we have been focusing on the structure, evolution and function of *PARP* genes, which encode a family of the poly(ADP-ribose) polymerases. The best studied family member, PARP-1, is an abundant nuclear enzyme, which can, upon activation, modify its target chromatin proteins by the synthesis of ADP-ribose polymer chains. PARP-1 is involved in the regulation of DNA repair, chromatin structure, transcription, cell death, and in the maintenance of genomic stability. The ongoing characterization of some other PARP family members suggests even more diverse roles for poly(ADP-ribosyl)ation in eukaryotes. (For recent reviews see Smith, Trends Biochem. Sci. 26: 174-179, 2001; Herceg and Wang, Mutat. Res. 477: 97-110, 2001; Kraus and Lis, Cell 113: 677-683, 2003.) One of the less studied PARPs is PARP-3 (Johansson, Genomics 57: 442-445, 1999; Augustin et al., J. Cell Sci. 116: 1551-1562, 2003).

Results

We characterized the mouse *PARP-3* gene encoding poly(ADP-ribose) polymerase-3 and the closely linked *U3-55k* gene coding for the U3 small nucleolar ribonucleoprotein complex-associated 55-kilodalton protein (1). Using a panel of mRNAs from various adult mouse tissues and organs, the expression profiles of *PARP-3*, *U3-55k*, *PARP-2* and *PARP-1* genes were determined (1). *PARP-3* expression exhibited marked differences among the individual tissues and some degree of tissue specificity, ranging from the highest transcript levels in the skeletal muscle to the barely detectable expression in the whole-brain and testis mRNA. In contrast to *PARP-3*, the other three genes were expressed ubiquitously, with less variability of mRNA levels.

Mouse *PARP-3* and *U3-55k* genes are located on chromosome 9 in a head-to-head orientation, being linked by an approximately 1.5-kb putative bi-directional promoter region (1). This gene arrangement has been conserved between mouse and human orthologues. Interestingly, the mouse and human *PARP-2* gene has been shown to be connected by a bi-directional promoter with the gene for the RNase P RNA subunit (Amé et al., J. Biol. Chem. 276: 11092-11099, 2001). As both the U3-55k protein and the RNase P RNA are involved in the processing of precursor RNAs of the protein-synthesizing machinery (pre-rRNA and pre-tRNA, respectively), we hypothesized that there might be a functional reason for the head-to-head coupling between some *PARP* and some protein-synthesizing machinery RNA-processing genes. For example, the expression of the two groups of genes might be coordinately regulated under certain physiological or pathological conditions and/or in some cell types (1).

Our work was supported by the Academy of Sciences of the Czech Republic (grant No. A5052802).

Publication

1. Urbánek, P., Pačes, J., Králová, J., Dvořák, M., Pačes, V.: Cloning and expression of *PARP-3* (*Adprt3*) and *U3-55k*, two genes closely linked on mouse chromosome 9. *Folia Biol. (Praha) 48: 182-191, 2002.*

Molecular Glycobiology

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[1] Members of the Center for Molecular and Gene Biotechnology, established and granted by the Ministry of Education, Youth and Sport of the Czech Republic.

Introduction

A major research effort of our laboratories is focused on studies of mammalian galectins, soluble β-galactoside-binding lectins, which participate in many biological processes such as development, immune response, signal transduction or malignancy. Another traditional part of our research is dealing with mammalian glycosyltransferases that control terminal oligosaccharide biosynthesis and carbohydrate interactions.

The Department forms an integral part of the "Center for Molecular and Gene Biotechnology", oriented towards the rapid development of modern biotechnologies with molecular genetics approaches for practical use in human and veterinary medicine production and their transfer to technology for final domestic producers. The Center, which has been formed out of groups from two Academy Institutes and the University, as well as from five home biomedical companies, is dealing with transgenic/gene-ablation biotechnologies for production of genetically modified animals, development of individual forms of recombinant proteins and on development of environmental biotechnologies.

Another part of applied research performed by our laboratories in collaboration with Biopharm-Institute for Biopharmacy and Veterinary Drugs, Inc., granted by the Ministry of Industry and Trade of the Czech Republic, was focused on production of commercially attractive recombinant proteins in body fluids of transgenic animals.

In the period of the last two years we also constructed several types of SHR-transgenic rats, representing modified models of spontaneously hypertensive rat (SHR) (collaboration with the Institute of Physiology AS CR, Prague, see references 2).

Results

We have studied the biological significance of selectin ligands and ICAM-1 for the onset of acute allograft rejection. Using a model of heterotopically transplanted heart of mice with a disrupted gene for ICAM-1 and/or Fuc-TVII we have shown that ICAM-1 deficiency leads to a partial delay in allograft rejection. Interestingly, the defect in the *Fuc-TVII* gene does not affect cardiac allograft rejection, yet this gene is normally upregulated in the activated vascular endothelium and Fuc-TVII participates in

inducible L-selectin ligand biosynthesis. When we transplanted hearts of ICAM-1 -/- mice into Fuc-TVII -/- recipients, the graft survival was significantly prolonged. Our data suggest the compensatory role of other receptor-counter-receptor pairs during acute allograft rejection and demonstrate that a cumulation of defects in interactions of adhesion molecules can significantly affect the onset of an organ rejection (see reference 1, in collaboration with the Institute for Clinical and Experimental Medicine, Prague).

In an effort to contribute to the understanding of the biological function of divalent monomer galectins, we cloned cDNA for mouse galectin-4 (GenBank Access No. AY044870) and used it for construction of bacterial plasmid vectors for recombinant galectin production. Those include natural as well as 6xHis-modified galectin-4 recombinant forms and N- and C-terminal recombinant carbohydrate recognition domains. These molecular probes represent an ideal tool for studies of the structure/function relationship and will be useful in searching for galectin-4 high-affinity ligands, crystallographic studies and the development of anti-galectin-4 specific antibody. We also investigated galectin-4 expression in different cell types and tissues using RT-PCR and *in situ* hybridization techniques. We were also able to isolate and sequence the purified galectin-4 genomic locus and to construct „transgenic" and „gene targeting" galectin-4 DNA vectors.

Regarding the applied research, in collaboration with Biopharm-Institute for Biopharmacy and Veterinary Drugs, Inc., we generated transgenic rabbits secreting the biologically active form of human erythropoietin in the milk. Transgenic females produce a high level of the transgenic product during the lactation period and transmit the transgene to the offspring. Apart from the commercial utility, this developed transgenic rabbit model represents an excellent source for studies of the glycosylation pattern of human erythropoietin produced in the animal body.

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 304/03/0090, 301/00/1636, 301/01/0278), Grant Agency of the Ministry of Health of the Czech Republic (research grant No. NB6468-3/2001), and Grant Agency of the Ministry of Industry and Trade of the Czech Republic (research grant No. FD-K/052/01). The Center for Molecular and Gene Biotechnology is being supported by the Grant Agency of the Ministry of Education, Youth and Sports of the Czech Republic (project No. LN00B030).

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Cell Differentiation

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[1] Supported in part by Faculty of Science, Charles University, Prague, and by the Grant Agency of the Czech Republic (204/03/H066)

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 haematopoietic 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. Finally, we studied the prolyl-isomerase Pin1, an important mediator of phosphorylation-dependent signalling.

Our laboratory closely collaborated with the group of Michal Dvořák (Molecular Virology, IMG) and with Martin Zenke (MDC, Berlin, 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 TRα/c-erbA at higher than endogenous levels. TRα/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 the selected targets to study their biological relevance. This project was carried out in collaboration with Petr Pajer (Department of Molecular Virology) and Martin Zenke (MDC, Berlin, Germany).

In addition to these studies utilizing the chicken model, we have collaborated with the laboratory of Martin Zenke (MDC, Berlin, Germany) to search for genes regulated by T3 during normal human erythroid differentiation employing Affymetrix DNA chips (Koh et al., in preparation).

Pin1 is a recently discovered small peptidyl-prolyl cis/trans isomerase with the ability to bind and isomerize certain phosphorylated Ser/Thr-Pro bonds in the proteins; many of them are

involved in the regulation of the cell-cycle progression. We have prepared all essential tools, including retroviral constructs, purified recombinant proteins and antibodies, that will enable us to study the role of Pin1 in cell proliferation and differentiation or leukaemia formation.

We continued to study the role of Myb proteins in cell fate determination of haematopoietic cells. We have demonstrated that bFGF, in cooperation with Myb proteins, represents an important factor for determining the erythroid lineage choice and supports progenitor proliferation (1).

GATA-1 and c-Myb transcription factors represent key regulators of red blood cell development. GATA-1 is upregulated and c-*myb* proto-oncogene expression is downregulated when red cell progenitors differentiate into erythrocytes. We have shown that c-*myb* proto-oncogene expression is effectively downregulated at the time when nuclear GATA-1 accumulates and cells differentiate into mature erythrocytes. Additionally, we have identified GATA-1 binding sites within the c-*myb* promoter and demonstrated that the GATA-1 protein binds to these sites *in vitro*. Furthermore, GATA-1 represses c-*myb* expression through one of the GATA-1 binding sites in transient transfection experiments and this requires FOG-1. We thus provided evidence for a direct molecular link between GATA-1 activity and c-*myb* proto-oncogene expression during terminal red cell differentiation (3).

The work of the group was supported by the VolkswagenStiftung (grant No. I/77 849).

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Cell Signalling and Apoptosis

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Introduction

Research in our laboratory is focused on the membrane receptors-born cell signalling pathways that play a role in cell growth, differentiation and apoptosis. One of our major projects addresses the mechanism, regulation and possible practical exploration of signalling from the group of "death receptors" of the TNFR (tumour necrosis factor receptor) family, mainly from receptors for TRAIL (TNF-related apoptosis-inducing ligand) and death receptor 6 (DR6). These receptors contain a protein-protein interaction region in their intracellular part called the death domain and the major outcome of their signalling is induction of apoptosis. However, their triggering also leads to activation of SAP and MAP kinases and NFkB and therefore possibly also to non-apoptotic signalling. We also investigate the role of the adaptor protein Daxx in the tuning of apoptotic signalling from these receptors. 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 implicated in cellular transformation and cancer. The interaction of extracellular Wnt ligands with the Frizzled/LRP receptor complex results in increased intracellular levels of β-catenin in the target cell. β-catenin then translocates into the nucleus and in complex with TCF/LEF proteins activates the expression of specific Wnt responsive genes such as c-*myc*, cyclin D1, and *Pitx2*. We search for proteins that participate in this Wnt-TCF signalling axis and we analyse the interplay between the Wnt and other cell signalling pathways.

Results

We have characterized the possible mechanism of CD43 crosslinking-induced apoptosis of erythro-myeloid cell line TF-1 and found that CD43-interacting adaptor protein Daxx partly inhibits this apoptosis (1). We have also prepared several variants of the soluble cytotoxic ligand TRAIL and showed that it alone and in combination with a novel chemotherapeutical agent α-tocopherol succinate (α-TOS) efficiently induces apoptosis of a number of tumour-derived cell lines and primary leukaemia cells (2-5). Currently we investigate the mechanism of TRAIL-induced apoptosis and DR6-activated signalling and characterize apoptosis-related activities of the adaptor protein Daxx. 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 (6). At present, we analyse the role of other Tcf-4-interacting proteins in Wnt-induced signalling.

The work of our group was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (research grant A5052304), and by the Ministry of Education, Youth and Sports of the Czech Republic (Center of Molecular and Cellular Immunology LN00A0026 and Eureka OE138 RETRAIL).

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Signal Transduction

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[1] Supported by1st Medical Faculty, Charles University, Prague
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Introduction

Binding of multivalent ligands to surface receptors induces receptor aggregation and subsequent biochemical pathways leading to cell activation. Our laboratory has been traditionally dealing with analysis of early molecular events in activated mast cells. These cells can be triggered by aggregation of the high affinity receptor for IgE (FcεRI). Binding of multivalent antigen to IgE anchored to FcεRI results in rapid tyrosine phosphorylation of the β and γ subunits of the receptor and other signal transduction molecules in seconds after triggering. This is followed by activation and translocation of numerous signalling molecules leading to secretion of preformed mediators of allergy reactions (e. g. histamine) in minutes after cell triggering. More than a decade ago we discovered that mast cells could also be activated by aggregation of glycosylphosphatidyl-inositol (GPI)-anchored protein Thy-1. Further studies indicated that Thy-1 and other GPI-anchored proteins are located in glycosphingolipid- and cholesterol-enriched plasma membrane microdomains, called lipid rafts. These microdomains have been suggested to play an important role in signalling not only via GPI-anchored proteins, but also other surface receptors, including FcεRI. We are interested in understanding the composition and functional properties of these membrane microdomains in the course of mast cell activation.

Results

In order to better understand the complexity of lipid raft components, we have prepared monoclonal antibodies against lipid rafts isolated from a model mast cell line (RBL-2H3). One of the antibodies was found to recognize a novel GPI-anchored plasma membrane glycoprotein of 250 amino acids, designated TEC-21, containing a cysteine-rich domain homologous to urokinase-plasminogen activator receptor/Ly-6/snake neurotoxin family. TEC-21 was found to be abundant on the surface of RBL-2H3 cells, but was absent in numerous rat tissues except for testes. Aggregation of TEC-21 induced a rapid increase in tyrosine phosphorylation of several substrates including Syk kinase and LAT adaptor, calcium

flux, and release of secretory components. However, aggregation of TEC-21 did not induce changes in the density of IgE-FcεRI complexes, tyrosine phosphorylation of FcεRI β and γ subunits and co-aggregation of Lyn kinase. The combined data indicate that TEC-21 is a novel lipid raft component of RBL-2H3 cells, whose aggregation induces cell activation independently of FcεRI (1).

In collaboration with the Laboratory of Leukocyte Antigens we studied the properties of a novel lipid raft transmembrane adapter protein NTAL (non-T cell activation linker). We have found that NTAL, which is located in lipid rafts, is rapidly tyrosine-phosphorylated upon FcεRI aggregation in both RBL and bone marrow-derived mast cells (2).

We have also analysed the molecular mechanisms of the inhibitory effect of gangliosides on FcεRI-mediated mast cell activation. We have found that pretreatment of RBL-2H3 cells with isolated brain gangliosides inhibited degranulation of cells activated via FcεRI but not Thy-1 glycoprotein. Exogenously administered gangliosides also inhibited formation of filamentous actin and production of phosphoinositides. Gangliosides had no or only a marginal effect on the association of aggregated FcεRI with lipid rafts, on tyrosine phosphorylation of FcεRI and LAT adaptor. Though pretreatment with gangliosides did not inhibit the association of LAT with phospholipase C (PLC)γ1 and PLCγ2, tyrosine phosphorylation of these enzymes, as well as their enzymatic activities and association with detergent-insoluble signalling assemblies, were reduced. This resulted in a decreased production of inositol 1,4,5-trisphosphate and inhibition of Ca^{2+} mobilization. These data support the concept that exogenously administered gangliosides interfere with those properties of GEM that are important for the formation of plasma membrane-associated signalling assemblies containing PLCγ but not for initial tyrosine phosphorylation of FcεRI subunits (3).

Other studies involved the discovery of the Fyn kinase-dependent pathway in FcεRI-activated mast cells (4), development of novel one-tube semi-nested PCR-ELISA (5), analysis of an endocrine disruptor on mammalian fertility (6), assessment of the inhibitory effect of anticoagulant drugs and cimetidine on cancer cell adhesion (7).

We have also prepared review articles where we scrutinized recent data on the role of non-receptor protein tyrosine and lipid phosphatases (8) and lipid rafts (9) in mast cell signalling.

The work of the group was supported by the Grant Agency of the Ministry of Education, Youth and Sports of the Czech Republic (research grant No. LN00A026), Grant Agency of the Czech Republic (research grants Nos. 204/00/0204, 204/03/0594, 310/00/205, 301/03/0596), Grant Agency of the Academy of Sciences of the Czech Republic (research grants Nos. A5052005/00, A7052006/00, A50582310 and S5052201), Grant Agency of the Ministry of Health of the Czech Republic (research grant No. NB/6758-3), and by the Howard Hughes Medical Institute (research grant No. 55000304).

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Intracellular Communications

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Technicians: Miloslava Čechová, Iva Dosoudilová

Introduction

Several signal transduction pathways are deregulated in human diseases such as diabetes and cancer. This is associated with changes in the activity of signalling proteins including signal transducers and activators of protein kinase cascades. Activation of protein kinase cascades is an efficient regulatory mechanism, allowing a rapid and specific response of cells even to small changes in extracellular and intracellular signals. To characterize the processes that may be responsible for malignant transformation of cells, we have focused our studies on the molecular mechanisms implicated in intracellular signalling in tumour and normal cells. In particular, we analysed human colon cancer cells **(A)** and the 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 of inducing 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 markers in the mitogenic and oncogenic potential of tumour cells. One of these markers, β-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. β-catenin is a substrate for several protein kinases and its phosphorylation catalyzed by glycogen synthase kinase-3 (GSK-3), a key protein effector in the phosphoinositol 3-kinase (PI-3K)/Akt/GSK-3 signalling pathway, results in β-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 types of cells, E-cadherin can induce ligand-independent activation of the EGF receptor (EGFR), probably by means of their co-clustering. The state and activation of EGFRs were therefore analysed in colorectal cell lines. A possible role of PKCβII in proliferation and differentiation of colon cancer cells was also examined.

(B) Increased protein synthesis is necessary for cancer progression to support a rapid proliferation of cancer cells. A major mechanism regulating protein biosynthesis is translational control, which is mediated by the interactions between cell signalling and components of the translational apparatus. In addition to a global translational control that regulates the cell's response to external stimuli such as growth factors, cytokines, stress and viral infections, selective translational control has been demonstrated to affect several genes related to growth, cell progression and apoptotic processes. These mRNAs are poorly translated in quiescent cells, but preferably recruited to ribosomes after a mitogenic signal. The main factors contributing to the low efficiency of mRNA translation include

structural elements within particular mRNAs, such as the presence of a polypyrimidine-rich motif (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, controlled by the rapamycin-sensitive signalling pathway of mTOR. 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 translating polysomes. By controlling cap-dependent translation and the translation of 5'TOP mRNA, the signalling pathway of mTOR upregulates the translational machinery and increases the translational capacity of the cell under favourable growth conditions. This may result in an increase in the rate of protein synthesis caused by an additional selective increase in the translation of such specific subset of mRNAs that are normally found in a translationally repressed state. Their enhanced translation may induce malignant transformation or alter the cell growth.

Another important signalling pathway that is activated by growth-promoting stimuli is the rapamycin-insensitive pathway, which is implicated in both cell survival and death by activating multiple distinct downstream signalling cascades. Activation of the lipid kinase, phosphoinositide-3 kinase (PI-3K), leads to the activation of serine/threonine protein kinases including PDK1, PKB/Akt, p70 S6K, and inactivation of GSK-3.

To explore the mechanisms that control mRNA translation and may be involved in the process of cell malignancy, we have analysed hamster fibroblasts that were transformed by Rous sarcoma virus (RSV). Examination of cellular responses to the activated Src protein, which is overexpressed in RSV-transformed cells, may help to recognize 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 depends on the time of exposure and glucose deprivation. While the prolonged butyrate treatment was followed with a decrease in the activity of alkaline phosphatase, a high additive effect on this activity was found after butyrate treatment of glucose-starved cells. Thus, the absence of this energetic source substantialy increases the differentiation effect of butyrate (1). Alterations in mitochondria function and morphology in HT29 cells upon conditions inducing differentiation were also found. Our study confirmed the ability of butyrate treatment and glucose deprivation to induce a more differentiated phenotype with changes of the subcellular structures leading to apoptosis. The different types of mitochondria found in HT29 cells after their treatment with differentiation-inducing agents suggest the pleiotropic response of this cancer cell line, obviously depending on the differentiated state of the cells and particular culture conditions. Nevertheless, „normalization" of mitochondrial morphology in a great part of the treated cells coincided with the biochemical markers of differentiation (2). Upon EGF treatment, we have observed activation of the PI3-K/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.

(B) We have shown previously 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 the upregulation of protein biosynthesis, which is accompanied with the activation of at least two distinct signalling

pathways. We have found that approximately 20 % of general protein synthesis in v-*src*-transformed cells represents synthesis of proteins encoded by a specific subset of mRNAs that are translationally controlled by rapamycin-sensitive mechanisms of the p70 S6K and the repressor protein 4E-BP1, which are upregulated by the mTOR signalling pathway.

Another 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.

RSV-transformed fibroblasts show a high basal activity of PI-3K. This is reflected in a high basal activity of PKB/Akt, decreased activity of its downstream effector GSK-3 and increased GDP/GTP exchange activity of the translation factor eIF2B, the substrate of GSK-3. eIF2B mediates the recycling of initiation factor eIF2, which is required for every initiation cycle. Therefore, the activity of eIF2B may be involved in the overall activation of translation.

A high basal activity of p70 S6K and the increased phosphorylation of its physiological substrate, 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. On the other hand, 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 (3).

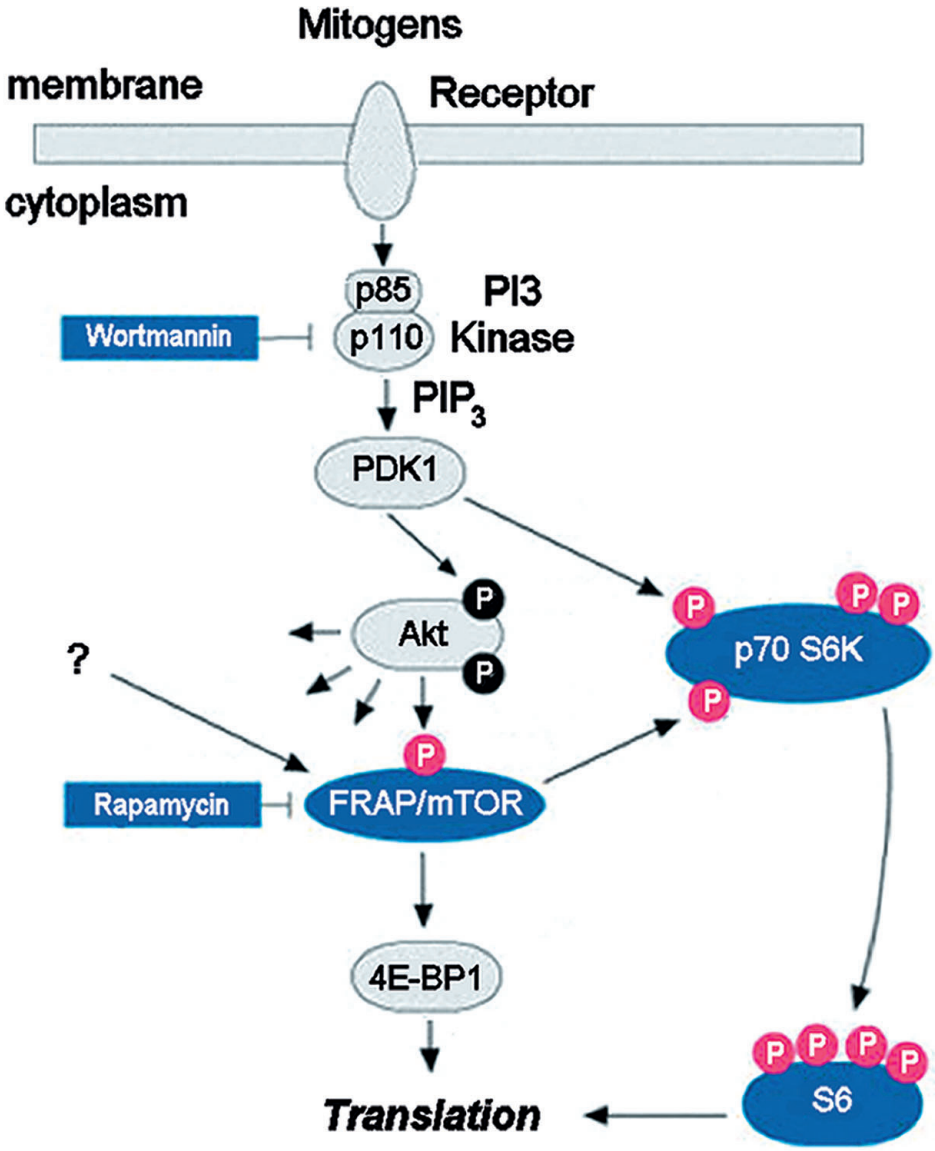
Our studies thus revealed two important differences between v-*src*-transformed and non-transformed cells in respect of the regulation of proteins that modulate selective and global protein synthesis and the signalling events that impinge upon them. We have found that at least two signalling pathways that control mRNA translation are activated by RSV transformation of hamster fibroblasts. The first is the rapamycin-sensitive mTOR signalling pathway, which regulates the phosphorylation and activity of the ribosomal S6 protein kinase p70 S6K and the phosphorylation of translational repressor protein 4E-BP1, which is independent of PI-3K in v-*src*-transformed cells. The second is a distinct antiapoptotic PI-3K/Akt/GSK-3 signalling pathway that increases the activity of the regulatory initiation factor eIF2B.

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 (5,6). 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.

Phosphorylation of the S6 protein of 40S ribosomal subunit, which is responsible for binding and decoding mRNA, is a common effect of mitogens proposed to regulate 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.

These data indicate that, unlike in v-*src*-transformed cells, the PI-3K signalling pathway in concert with the signalling pathway of mTOR is involved in IL2-dependent phosphorylation of the ribosomal protein S6 (7).

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 301/00/0269 and 301/02/D159) and the Grant Agency of the Academy of Sciences of the Czech Republic (research grant No. 3KJB5052302).


Mitogens
membrane
Receptor
cytoplasm
p85
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Kinase
Wortmannin
PIP3
PDK1
P
Akt
?
p70 S6K
Rapamycin
FRAP/mTOR
4E-BP1
Translation
S6

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Biology of Cytoskeleton

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Introduction

The long-term research programme of the laboratory has been to study 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 αβ-tubulin heterodimes and a collection of microtubule-associated proteins (MAPs). Both tubulin subunits are coded 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 centers (MTOCs). One of the key components of MTOCs is γ-tubulin, which is necessary for nucleation and organization of microtubules. γ-tubulin also exists in cells in the form of soluble γ-tubulin complexes, whose composition and functions start to be identified. Recent data indicate that γ-tubulin could have other functions than nucleation of microtubules. We have focused our efforts mainly on the elucidation of the distribution and function of γ-tubulin in different cell types.

Results

To gain insights into the function of γ-tubulin in microtubule marginal band formation in differentiating erythroid cells, we have followed its distribution in developing chicken erythrocytes and characterized the soluble forms of the protein. Subcellular localization of γ-tubulin has changed in the course of differentiation, and in postnatal peripheral erythrocytes γ-tubulin was found only in soluble forms. γ-tubulin occurred in multiple charge variants, whose number increased in the course of differentiation. Combined data indicated for the first time that the properties of γ-tubulin or its complexes change during differentiation events, and that γ-tubulin is a substrate for developmentally regulated post-translational modifications (1).

We have shown that there are two γ-tubulin forms in the brain that are present in complexes of various sizes. Large complexes tend to dissociate in the presence of high-salt concentration. Both γ-tubulins copolymerized with tubulin dimers, and multiple γ-tubulin bands were identified in microtubule protein preparations under conditions of non-denaturing electrophoresis. These data indicate that γ-tubulin itself and/or γ-tubulin with another unidentified protein has

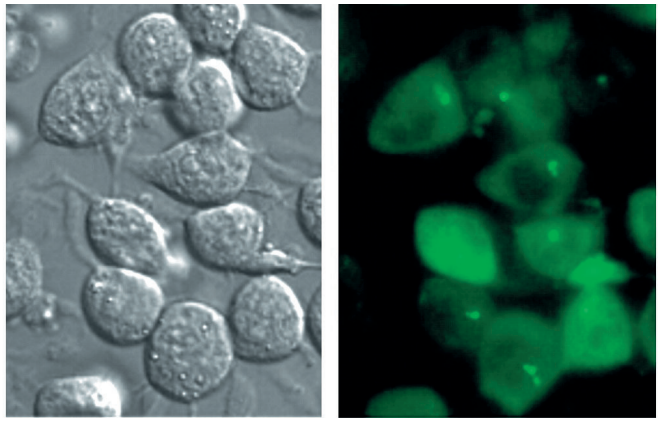

Fig. 1. Live cell imaging of EGFP-tagged gamma-tubulin in rat basophilic leukaemia cells RBL.

the ability to form oligomers. Immunoprecipitation experiments with monoclonal antibodies against γ-tubulin and α-tubulin revealed interactions of both γ-tubulin forms with tubulin dimers irrespective of the size of complexes. We suggest that besides small and large γ-tubulin complexes, other molecular γ-tubulin form(s) exist (3, 6, 8).

Association of γ-tubulin with tubulin dimers was not limited to vertebrates, but it was also observed in acentriolar plant cells that contain a large soluble pool of γ-tubulin. In these cells, large γ-tubulin complexes, resistant to salt treatment, were found to be associated with a high-speed microsomal fraction. Blue native electrophoresis of detergent-solubilized microsomes showed that the molecular mass of the complexes was >1 MD. Large γ-tubulin complexes were active in microtubule nucleation, but the nucleation activity was not observed for the smaller complexes. Immunolocalization revealed accumulation of γ-tubulin on short kinetochore microtubules interacting in polar regions with membranes. Our results indicate that the association of γ-tubulin complexes with dynamic membranes might ensure the flexibility of non-centrosomal microtubule nucleation (5, 7). We have also collected data on a complex distribution pattern of γ-tubulin in Leishmania. The cell cycle-dependent distribution of γ-tubulin in the posterior end of the cell could imply its important role in microtubule anchorage during interphase and mitosis (12).

Using a panel of well-characterized antibodies against neurofilaments (NF) we have found that phosphorylated high-molecular-weight protein (NF-H) accumulates in subsets of degenerating neurons during aging. This points to a susceptibility of NF to phosphorylation-related morphological changes during neurodegeneration (9). We have also found that glial fibrillary acidic protein (GFAP) can be used as an additional diagnostic tool in the evaluation of Alzheimer's disease (10). New monoclonal antibodies against kinesin (2, 4) and clathrin (11) were generated.

The work of the group was supported by the Grant Agency of the Czech Republic (Research Grant No. 304/00/0553), the Grant Agency of the Academy of Sciences of the Czech Republic (Research Grants Nos. A5052004, IBS5052301), by grants from the Ministry of Education (OE 68/1, ME-310) and by project LN00A026 (Center of Molecular and Cellular Immunology) from the Ministry of Education.

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Cell Biology

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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 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 enhanced healing of burns and other skin defects
2) human tumour and normal tissues: primary cell isolation, cultivation and characterization in relationship to the neoplastic disease
3) chemosensitivity/chemoresistance: evaluation of individual breast tumours
4) fast intracellular motion (FIM): analysis of the phenomenon and its indicative potential, development of quantification of series of fuzzy images, and search for a widely available detection method.
5) rat sarcoma cells: dynamic morphotype *in vitro* as related to malignancy *in vivo*
6) intestinal cells as target cells for HIV infection *in vitro*

Results

Deep dermal burns frequently tend to convert into full-thickness skin loss. We have 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 (1). RHPS, which shows a skin-like consistency and therefore optimal adhesiveness to the wound, has to be applied on the defect with the keratinocyte layer facing the wound. The wound must be prepared by tangential excision or dermabrasion to the level of capillary bleeding. In our practice, more than 70%

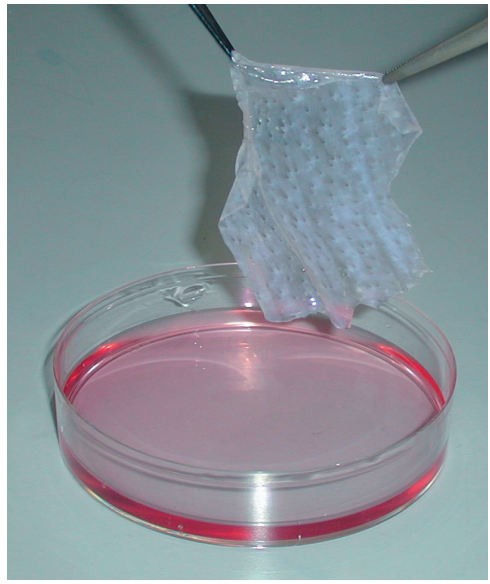

Fig. 1. RHPS is composed of human allogeneic keratinocytes cultured on cell-free pig dermis. Its consistency is similar to the normal skin.

of early excised or deeply dermabraded wounds covered with the recombined skin healed in the course of one week after RHPS grafting (Fig.1, Fig. 2a,b) (1).

Breast cancer belongs to poorly understood human cancers. One of the reasons is the 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 epithelial cells (including the luminal phenotype) from small biopsies of human breast tumours and cutaneous metastases. A feeder layer of irradiated NIH 3T3 cells was used for cultivation. Forty-one out of 47 primary tumour specimens and all of the three cutaneous metastases grew successfully for 2-10 passages *in vitro*. In the majority of cultured cell populations, a fraction of luminal cells positive for keratin 19 (K19+), supposed to be the cell type from which malignant cells arise, was detected. Cultivation of neoplastic cells was indicated by positive immunostaining for the p53 oncoprotein, the Src oncoprotein, and overexpression of the HER-2/neu (2).

The low cellular yield of a breast cancer sample is a limiting factor for *in vitro* chemosensitivity/chemoresistance testing. The use of *in vitro* serially cultured cells can help overcome this obstacle. *In vitro* drug resistance of cells cultured from mammary carcinomas by the 3T3 feeder-layer technique was tested by the MTT assay. Out of the 33 tested cultures, 9 were derived from cells obtained from true-cut biopsies of primary

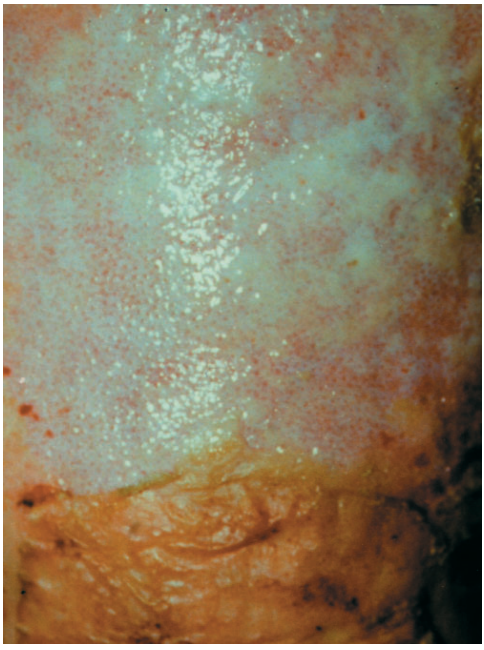

Fig. 2a. A 3-year-old boy was scalded on 30% of the body surface. A part of the wound was tangentially excised and covered with RHPS (day 2 after grafting).

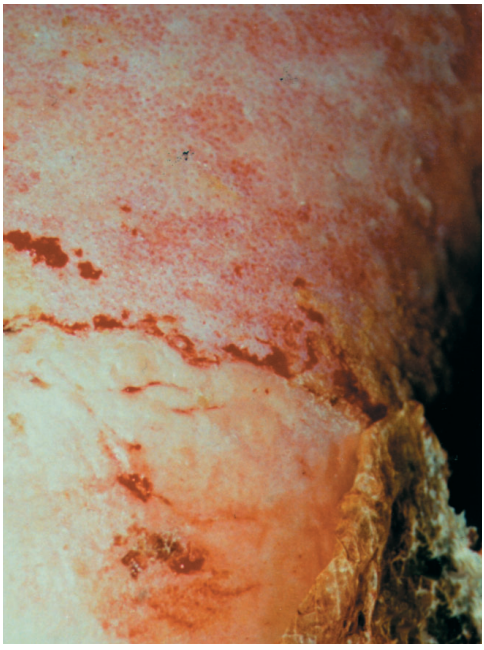

Fig. 2b. Five days after grafting, the RHPS was mechanically peeled off, leaving newly formed epithelium underneath. The second part of the wound had to be autografted.

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tumours, with sample volume less than 0.03 cm^3. The cultures were treated with six anticancer drugs. The chemoresistance of cultured cells was monitored by the surviving cell fraction. Paclitaxel and cisplatin were the most potent drugs. Gemcitabine, vinorelbine and mafosfamide were the least potent drugs. Doxorubicin and gemcitabine most frequently failed to completely metabolically inhibit 100% of cultured cells. Combination of the optimized feeder-layer cultivation technique and the MTT test permitted extensive drug resistance testing from very small breast cancer samples (3).

Fast intracellular motion (FIM) was revealed due to highest spatial and temporal resolution of back scattered light (BSL) imaging provided by Video Rate Confocal Laser Scanning Microscope (VRCLSM) Odyssey (Noran, Middleton, WI, USA). A search for a suitable method for quantification of FIM led to correlation analysis calibrated on Brownian motion and a known type of motion such as cell marginal ruffling. This opened the way for comparing cellular activities in terms of velocity. This is particularly interesting for investigation of neoplastic versus normal cells. It is also a way of assessment of alternative methods for visualization of FIM, which can then become widely available (4).

The dynamic morphotype of tumour cells was studied as a conjunction between cell shape and migration. This enabled the investigation of the relationship between malignancy and patterns of dynamic morphology of neoplastic cells *in vitro*. Time-lapse cinemicroscopy was used to analyse the cell behaviour of three rat neoplastic cell lines, differing in the metastatic potential. These populations were studied for incidence of the dynamic morphotypes in culture media differing in pH. The results showed that acid pH stimulated the motile activity in highly malignant cells and revealed two fast moving dynamic morphotypes associated with a high metastatic potential (5).

Intestinal cells were cultured as target cells for HIV infection and analysed for surface HIV receptors and co-receptors. It was shown that adaptation of a CXCR4-using human immunodeficiency type 1 NDK virus in intestinal cells is associated with CD4-independent replication of NDK (collaboration with Dr. Ivan Hirsch, INSERM, Marseille) (6).

The group's work was supported by "The Programme 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 (Grant No. NC/6734-3), by the Czech Oncological Society J.E. Purkyně (Race of Terry Fox) and by the League against Cancer.

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Protein Biosynthesis

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Introduction

The long-term research programme of the laboratory involves two main topics:

(i) Structure and function of protein synthesis components and regulation of their genes in prokaryotes

(ii) Src family kinases and vertebrate development

In the last two years the research in the above fields has been dealing with (i) the elucidation of principles of themostabilization of molecules of elongation factors EF-Tu and with (ii) the localization of the c-*src* protooncogene on *X. laevis* chromosomes and the genomic structure of *Mus musculus* c-*src* protooncogene.

Ad (i) Elongation factors EF-Tu are monomeric proteins composed of about 400 amino acid residues. They are the most expressed proteins in prokaryotes, comprising 5-10% of the total cellular proteins, e.g. in *Escherichia coli.* EF-Tus are GTPases essential for mRNA translation on ribosomes in all known microorganisms. The proteins are highly homologous and folded into three clearly distinct domains. This predetermines EF-Tu factors for the study of structural features of adaptation of multidomain proteins to different living conditions. One of them is the adaptation to high temperatures. We compared EF-Tu from the mesophilic *E. coli*, growing at around 37˚C, and EF-Tu from thermophilic *Bacillus stearothermophilus*, growing at around 60˚C, to explore, by the domain chimerization approach (1,2), the common mechanisms of the thermal stabilization of EF-Tu molecules, and to derive the structural differences responsible for a lower thermal stability of *E. coli* EF-Tu in comparison to *B. stearothermophilus* EF-Tu.

Ad (ii) The c-Src enzyme is a member of the family of tyrosine kinases, which are ubiquitous in every higher animal cell. It has been implicated in the regulation of cell growth and differentiation and its retroviral homologue, the v-*src* of the Rous sarcoma virus (RSV), is a well-established oncogene. The involvement of Src in the development has been characterized only partially. To address this question we first prepared, by sperm-mediated DNA transfer technology, transgenic *Xenopus laevis* organisms expressing elevated levels of the kinase. The transgenic DNA applied consisted of the proviral form of the RSV genome carried on a plasmid. *X. laevis* was chosen as a model organism that allows an easy monitoring of all developmental stages *in vitro*. Transgenic frog embryos carrying integrated RSV LTR and v-*src* sequences in their DNA and expressing a high level of v-Src kinase in their tissues were characterized by defective morphogenesis. The RSV-LTR element(s) was transfer-

able onto the next generation of frogs. That time, however, an enhanced expression of the c-Src was detected. If it exceeded a certain threshold, it was accompanied with aberrant morphogenesis to a similar extent as in the case of the v-*src* transgenesis in the first transgenic generation (summarized in ref. 3). Expression of the c-Src below this threshold appeared to be phenotypically indifferent and tolerable during embryogenesis. Tissues of the defective frog embryos expressing the high level of c-Src were found to be strongly depleted of cadherin and α-, β-, γ-catenins, the adhesion molecules that mediate cell-cell contacts at adherens junctions (4). On the basis of these results we hypothesized that i) in the course of embryogenesis, the high c-Src can downregulate the cadherin-catenin adhesion molecules and ii) the high c-Src expression in tissues of the transgenic frog embryo of the second generation is the result of the activation of one or both *X. laevis* c-*src* genes by the integrated RSV-LTR element. Our experiments indicate that a high level of both v-Src and c-Src is not compatible with a normal frog embryogenesis and suggest that the adverse effects of the kinase on development might be due to the downregulation of the cadherin-catenin system.

Results

Mechanisms of thermal adaptation of proteosynthetic elongation factors EF-Tu

Six chimeric forms of EF-Tu, composed of different combinations of domains of mesophilic (*E. coli,* Ec) and thermophilic (*B. stearothermophilus,* Bst) EF-Tu, were prepared together with isolated recombinant domain 1 of both EF-Tus, and their binding, enzymatic and thermostability parameters were determined and compared with those of parental EF-Tus. Domain 1 of EF-Tu proteins is called the G-domain, because it binds GDP and GTP and has a GTPase activity. It represents the functional module of the EF-Tus.

Thermostability of the proteins was determined both functionally, as the preservation, at increasing temperature, of their functions (GDP/GTP binding and GTPase activity) and structurally, as the preservation, at increasing temperature, of their α-helix content (measured by CD spectroscopy).

Guanine-nucleotide binding experiments revealed that both EF-Tus as well as their chimeric forms bind GDP and GTP with a very similar affinity, which is about 100 times stronger for GDP than for GTP. This also applied to the isolated BstG-domain, it possessed affinities for GDP and GTP quite comparable with those of the whole EF-Tu. In contrast, the EcG-domain affinity for GDP and GTP was about 1000 times and 20 times, respectively, lower than were those of EcEF-Tu. These data imply that in BstEF-Tu, the high and differential affinity for GDP and GTP appears to be intrinsic to the G-domain itself, whereas in EcEF-Tu, the interaction of all three domains is required to establish this phenotype.

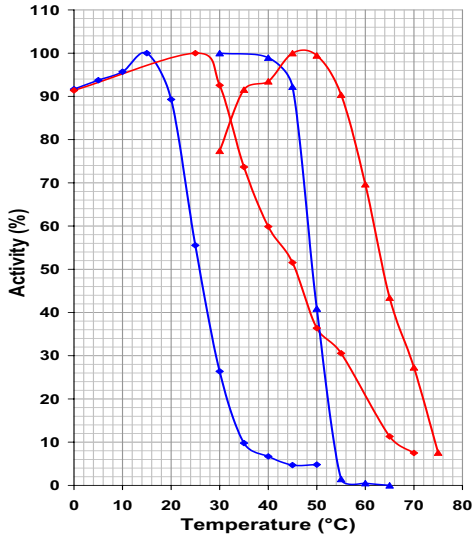

Fig. 1. Heat-inactivation profiles of GDP forms of *E. coli* (blue triangles) and *B. stearothermophilus* (red triangles) EF-Tus in comparison to those of *E. coli* (blue diamonds) and *B. stearothermophilus* (blue diamonds) G-domains

Thermal stabilization of EF-Tu of both *E. coli* and *B. stearothermophilus* was found to be consistent with a mechanism involving co-operative contributions from all three protein domains. The main determinants of the thermal stabilization were the catalytic G-domains (domains 1) of the proteins. They set up a „basic" level of the thermostability differently for either EF-Tu, about 20˚C below the optimal growth temperature of the respective organism. The thermostabilizing contributions of domains 2+3 were similar, much less influenced by their origin (Fig. 1). They consisted in stabilization of α-helical regions of the G-domains against thermal denaturation up to the level of the respective growth temperature optima of either organism (5).

The higher thermostability of the BstG-domain as compared to the EcG-domain could be attributed to two distinct structural features of the BstG-domain: (i) an increase, particularly on the G-domain surface, of charged residues at the expense of polar, uncharged residues (*C*v*P* bias), (Fig. 2) and (ii) a decrease in the nonpolar solvent-accessible surface area (5).

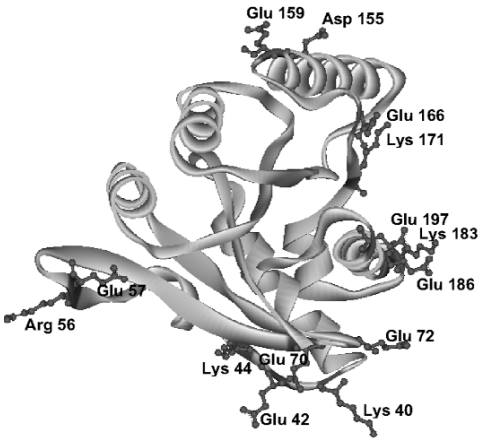

Fig. 2. Charged amino acid residues in the G-domain of EF-Tu of *B. stearothermophilus* newly introduced or different from those in the G-domain of EF-Tu of *E. coli*. This figure was made using the DS ViewerPro program.

Chromosomal localization of *X. laevis* c-*src* gene

This study was carried out in collaboration with the Department of Physiology and Developmental Biology of the Faculty of Science of Charles University in Prague.

To start elucidating the mechanism of the assumed activation effect of the integrated RSV-LTR element(s) on genomic c-*src* of *X. laevis* (see above), the chromosomal position of the *X. laevis* c-*src*1 gene was visualized (Fig. 3). As no suitable procedure for chromosomal localization of individual frog genes has been described yet, a new fluorescent *in situ* hybridization (FISH) protocol, including a tyramide amplification step (TSA), was developed. This is for the first time that a single copy gene was mapped on frog chromosomes (6). The c-*src*1 gene is located in the subcentromeric region of the long arm of one of acrocentric chromosomes of the G category.

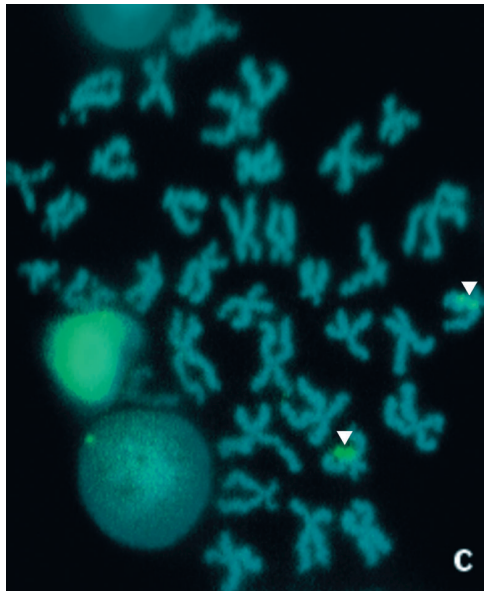

Fig. 3. Localization of the c-*src*1 gene (indicated by arrowheads) on the chromosomes of spleen cells of adult *Xenopus laevis* (FISH-TSA).

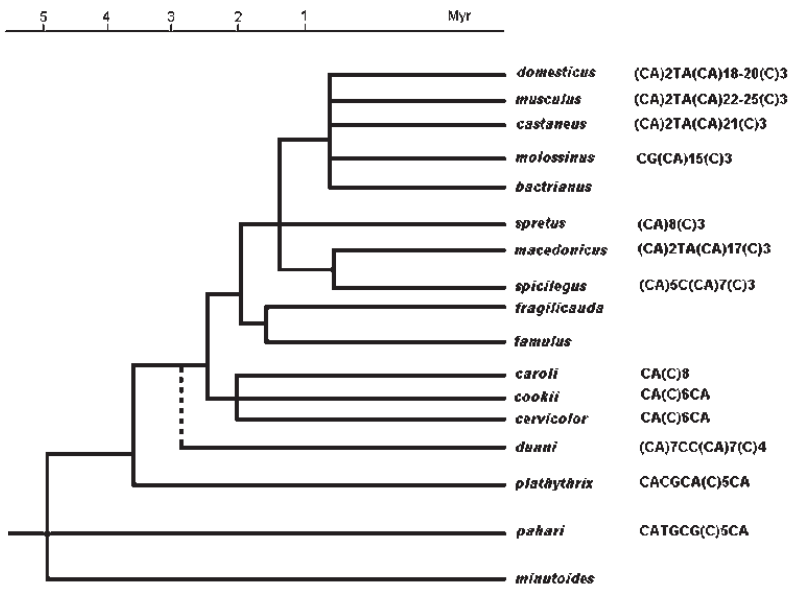

Fig. 4. Mouse taxonomic tree and the variability of the CA region of the microsatellite marker in the intron 5 of the mouse *src* protooncogene.

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

In the intron 5 of the mouse *src* protooncogene, a new microsatellite marker for chromosome 2, which was designated D2Img1, was discovered in our laboratory. It consisted of a CA dinucleotide run and 31 bp apart followed by a track of TG dinucleotides interrupted by extra Gs in specific intervals. As the 31-bp interlink can assume a hairpin structure, the whole region can be viewed as an imperfect inverted repeat (Fučík et al., 2000). Due to the conspicuous strain-, species- and subspecies-specific variations, this microsatellite can serve as a suitable taxonomic marker of mice from Europe and Asia (Fig. 4) and can contribute to the elucidation of intron evolution (Fučík et al., in preparation). In addition, the CA run appears to be subject to somatic mutations during ontogenesis. No similar structure is present in the corresponding human *src* intron.

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 301/02/0408 and 303/02/0689).

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Micromorphology of Biopolymers

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Introduction

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

(i) Ultrastructural and immunoelectron microscopical characterization of newly induced nucleolus-like structures in the nuclei of insect cells of the baculovirus expression system expressing the v-*myb* oncogene or the c-*myb* proto-oncogene.

(ii) The nucleolar architecture and interaction of cellular proteins with viral antigens were studied in the cells infected with mouse polyomavirus. We have focused on the analysis of the nuclear structure and processes taking place in the later stages of viral infection. The function of minor capsid proteins VP2 and VP3 localized in the „core" of polyomavirus was also analysed using different mutations of these proteins. Besides, the conformation of pentamers forming artificial pseudocapsids (a possible tool for gene therapy) was studied in different expression systems where mutated and nonmutated capsid proteins VP1 were synthesized.

(iii) Morphological analysis of colorectal adenocarcinoma cell line HT29 after treatment with natrium 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.

(iv) The influence of inhibitors mimosine and carbonyldiphosphonate on the deoxyribo-mode expression of primase activities of the Pr-α DNA polymerase enzyme complex belonging to the naturally occurring nucleoprotein complexes harbouring an extrachromosomal DNA was studied.

Results

The nucleolus-like structures in the nuclei of insect cells of the baculovirus expression system expressing the v-*myb* oncogene or the c-*myb* proto-oncogene resembled structurally large nucleoli typical for many cancer cells. They accumulated the Myb protein and contained DNA as detected by the immunogold DNA labelling method. They were also labelled with monoclonal antibody configuration-specific for immature RNA occuring in nucleoli. However, hybridization *in situ* and localization of fibrillarin indicated that these structures do not represent nucleoli functional in ribosomal biogenesis, but rather represent structures involved in viral replication and transcription (1).

Polyomavirus mutants E, Q and H expressing nonmyristylated VP2 were generated by replacing the A-terminal glycine residue with glutamic acid, glutamine or histidine, respectively. Viruses mutated in either VP2 or VP3 translation initiation codons were also prepared. All mutated genomes gave rise to viral particles. The infectivity of $VP2^{-}$ and $VP3^{-}$ viruses was dramatically diminished, indicative of defects in the early stages of infection. No differences in the number of cells expressing early or late viral antigens were observed between wt and E or Q myr^{-} viruses during the course of a life cycle (2).

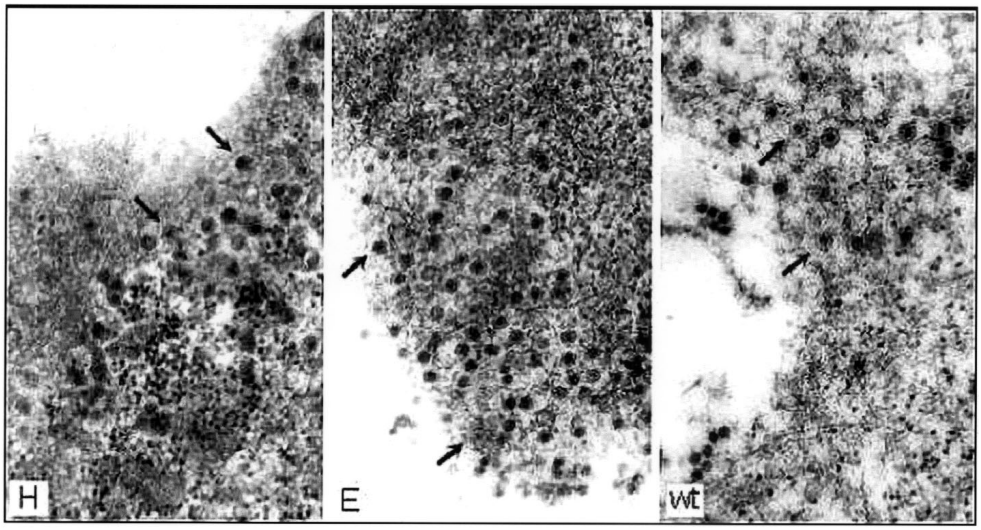

Fig. 1. Electron micrographs of mouse cells infected with mutated (H and E) or wild-type (wt) forms of polyoma virus (Magnification 100 000x)

Analyses of nucleolar structures revealed that infection with polyomavirus induces nucleolar volume increase and changes in the architecture of the cell nucleus. In the later stages of infection, the regions where transcription and replication of the viral genome takes place are separated from the areas of virion assembly and deposition. Formation of the virus progeny is accompanied with translocation of the YY1 protein.

The capsid protein VP1 mutated in the region for calcium binding (VP1 Ala) formed particles in both baculovirus or yeast expression systems. However, in comparison with particles containing nonmutated VP1, VP1 Ala pseudocapsids did not colocalize with tubulin and no affinity to structures resembling the mitotic spindle were observed. These findings suggest a changed surface conformation of pseudocapsids containing mutated VP1.

The short-term treatment of HT29 cells with sodium butyrate induced their differentiation (heavy brush border, orientation, specific cell conections, apoptosis, biochemical markers), whereas long treatment resulted in a decrease of some differentiation markers (3). Morphological and biochemical studies of HT29 cells treated with sodium butyrate and/or glucose-deprived revealed both apoptotic and differentiation response. The main apoptotic response was accompanied with an increase of floating cells. The ultrastructural analysis of adherent cells showed the typical apoptotic character of the nucleus in some of them. In addition, remarkable changes of mitochondria, assumed as early stages starting the apoptotic cascade, were observed. These changes were represented not only by alterations of mitochondrial morphology, but also by the number of mitochondria and their localization (6).

The deoxyribo-mode expression of primase (Pr) activities of the Pr-α DNA polymerase enzyme complex is similarly influenced by carbonyldiphosphonate (COMDP) as the ribo-mode expression of Pr activities. This compound strongly activates the deoxyribo-mode of Pr activities and again induces a unique phenomenon

of primer acumulation. These primers labelled for DNA are up to 90% alkali-resistant and sensitive to DNAse I treatment. In contrast to the stimulation of the ribo-mode expression of Pr activities by COMDP, the incorporated radioactivity is in this case more than one order lesser. 1-mimosine is again able to substantially eliminate the phenomenon of primer accumulation, suggesting that in this case the effects of COMDP and l-mimosine are also mutually exclusive and that both agents compete for the same active site responsible for mutual coupling of Pr and α DNA polymerase activities (4).

The members of the Micromorphology of Biopolymers group also participated as collaborators in other projects (5 and 7).

The work of the group was supported by the Grant Agency of the Czech Republic (research grants Nos. 204/00/0271, 204/03/0593), and by Howard Hughes Medical Institute Grant No. 75195-540501.

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Recombinant Expression and Structural Biology

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

Introduction

Structure and mechanism studies of non-active-site inhibitors of the HIV protease (HIV PR) form a "flag project" of this group. The retroviral proteinase of HIV is an attractive target for anti-viral 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 multipotent HIV PR active-site inhibitors developed at the Institute of Organic Chemistry and Biochemistry (ref. Rinnová et al., ABB 2000, *382*, 22-30). The inhibitors chosen for our crystallographic studies comprise compounds Z-Abt-Glu-Hph-NH_2; Z-Pns-Phe-Glu-Glu-NH_2; and Z-Pst-Glu-Hph-NH_2 having a picomolar range of their Ki values for the wild-type HIV PR as well as a various degree of insensitivity of their inhibitory potency to HIV PR mutations conferring Saquinavir, Ritonavir and Indinavir resistances.

The Department has well equipped biochemical, molecular cloning and protein purification and crystallization laboratories. Its experienced staff operates an x-ray diffractometer (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 interactions of certain oncogens, and into practically oriented protein design.

One of such research topics in the group is the structural characterization of the novel oncogenic MN/CA IX protein (briefly MN) with carbonic anhydrase activity, which is a cell surface protein expressed in a high percentage of certain human carcinomas (renal, cervical, lung, colorectal,

etc.). Our interest in MN and in the MN-specific antibody M75 is based on the expectation that MN can serve both as a diagnostic marker and a target molecule for therapy (ref. Závada J, et al., (2000), Br. J. Cancer. 82:1808-13). The monoclonal antibody M75 binds MN with high affinity. Using synthetic oligopeptides, the epitope recognized by mAb M75 was localized in the proteoglycan-like domain of CA IX, and identified as amino acid sequence PGEEDLP.

Results

The monoclonal antibody 1696, which was raised against the HIV-1 protease, inhibits the catalytic activity of the enzyme from both the HIV-1 and HIV-2 strains. The antibody cross-reacts with peptides containing the N-terminus of the enzyme, which is highly conserved between these strains. The crystal structure of a single-chain Fv fragment of 1696 (scFv-1696) in the non-complexed form, solved at 1.7 Å resolution, is compared with the previously reported non-complexed Fab-1696 and antigen-bound scFv-1696 structures. Large conformational changes in the third hypervariable region of the heavy chain and differences in relative orientation of the variable domains are observed between the different structures [1]. A review article has been published on the inhibition of HIV PR by monoclonal antibodies [2].

Complexes of HIV PR with novel active-site inhibitors from the series mentioned in the Introduction were successfully crystallized, and several structures were solved and refined. One of the complex structures was solved at 1.03 Å, the best resolution reported up to date for HIV PR complexes [3]. The x-ray structural and modelling results make it possible to suggest a structural basis of susceptibility of Ritonavir-resistant V82A HIV PR mutant to inhibition by a novel phenylnorstatine compound Z-Pns-Phe-Glu-Glu-NH_2 (cf. above). The results show, in brief, that the fit of the central part of the inhibitor to the P1 binding pocket of the mutant HIV PR is better

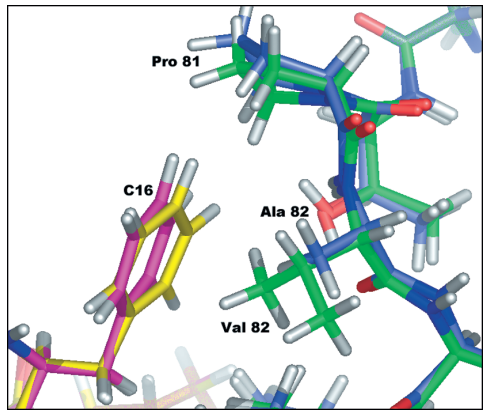

Fig. 1. Details of interactions of HIV PR and the Z-Pns-Phe-Glu-Glu-NH_2 inhibitor at the P1 subsite. The wild-type protein structure is coloured green, the modelled V82A mutant coloured blue. The inhibitor is coloured magenta for the wild-type structure, and yellow for the mutant model.

than that in the wild-type complex structure (Fig. 1). Owing to the relatively long "main-chain" of the critical central part of the inhibitor II, the occupation of the wild-type P1 pocket requires a deformation, as if the wild-type pocket was too "tight" for the respective aromatic "side-chain". All calculated parameters show that the strain is relieved with the V82A mutation. Such findings can be seen as a special case of the

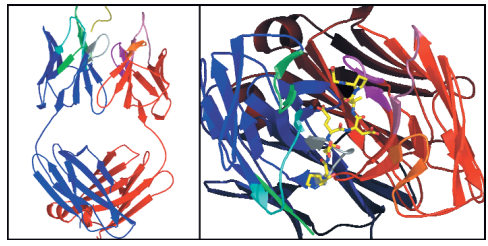

Fig. 2. Overall view of M75 Fab fragment structure (left panel) and detailed view of MN/CA-IX antigen peptide bound in the antigen binding cleft of the M75 Fab fragment (right panel, the peptide is colour-coded yellow).

contribution to the original ideas on the structural mechanisms of the HIV PR drug resistance.

Structural work has progressed on MN/CA IX-specific monoclonal antibody M75. We obtained crystals of free Fab M75 and of Fab M75 complexed with different variants of the epitope peptide.

The structure of free Fab M75 and Fab M75 in complex with peptide PGEEDLPGEEDL was solved and refined at 2.1 Å and 2.0 Å resolution, respectively [4].

The activities within the "Center for Molecular and Gene Biotechnology" represented the applied research. A recombinant viral antigen developed in the group became a component of approved commercial diagnostic kits for antibodies against the Epstein-Barr virus.

The international collaboration of this group was supported from the Czech Ministry of Education by the grant CZE-00-027 under the KONTAKT Programme and by the grant E!2245 under the EUREKA Programme, and from the C.E.C. 5th Programme, Concerted Action QLK2-CT-2001-02360. Local support included research grants from the Grant Agency of the Czech Republic, Nos. 203/98/K02 and 301/00/0689; from the Grant Agency of the Academy of Sciences of the Czech Republic, No. A4050811; and from the Czech Ministry of Industry and Commerce, FD-K3/001. The participation in the "Center for Molecular and Gene Biotechnology" was supported from the Czech Ministry of Education under code LN00B030.

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Biology and Biochemistry of Fertilization

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

Introduction

A. Biology and biochemistry of fertilization

Fertilization is a highly specialized interaction between gametes that culminates in formation of a zygote and in development of a new individual of the species.

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 motility, metabolism, capacitation and acrosome reaction are modulated by factors associated with the egg, its acellular or cellular investments, or fluids bathing both the male and female reproductive tracts. Sperm and zona pellucida proteins participate in the highly specialized interaction between gametes.

Many chemicals released into the environment can disrupt 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).

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

1. Traditional study of selected sperm proteins involved in the process of fertilization. In this connection, the role of selected cell surface and intra-acrosomal sperm proteins during capacitation was studied.
2. Effect of endocrine disruptors on mammalian fertility. Selected endocrine disruptors (present as food contaminants that constitute 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 served for detection of sperm damage. Monoclonal antibodies against intra-acrosomal sperm proteins were used as suitable and sensitive markers for detection of spermatogenesis in human medicine.

B. Cell growth control

The long-term research programme of the group is concerned with the mechanisms controlling proliferation and apoptosis in normal and tumour cells. Recently, we have mainly focused on (i) the

role of non-transferrin iron transport in cell growth control, on (ii) the mechanisms of apoptosis induction in tumour cells, and on (iii) the mechanisms of cell cycle control in tumour cells.

The acquisition of iron is critical for the growth of cells. Iron uptake via transferrin receptor-mediated endocytosis of transferrin has been relatively well described. However, the mechanism of non-transferrin iron transport across the plasma membrane still remains unclear in spite of the fact that recently a real progress in understanding the nature of non-transferrin iron transporters has been made. Growing tumour cells may utilize the uptake of non-transferrin iron as an alternative mechanism for acquiring iron. Therefore, we focused on characterization of non-transferrin iron uptake by various tumour cells. We have also focused on identification of membrane molecules involved in non-transferrin iron transport into cells.

Apoptosis, an evolutionarily highly conserved programme of cell self-destruction, plays a significant role in many physiological processes. It also occurs in tumour cells, including their response to therapy. There is an apparent interest in using iron deprivation as a tool of cancer therapy. Several papers demonstrate that iron deprivation could induce apoptosis. Employing DNA fragmentation analysis, flow cytometric analysis after propidium iodide staining and electron microscopy we have provided the first direct evidence that iron deprivation induces apoptosis in tumour cells. Recently, we have focused on the mechanism of apoptosis induction by iron deprivation. We have also focused on the mechanisms responsible for the resistance to apoptosis induction in tumour cells.

Accumulating data, including our findings, support the hypothesis that iron is involved in the control of the cell cycle. However, the available data still have a character of a fairly incomplete mosaic. Therefore, we decided to begin studies concerning the role of iron in the control of the cell cycle. At the beginning we have focused on the identification of molecules affecting the regulatory mechanism of the cell cycle under changed availability of iron.

Results

A. Biology and biochemistry of fertilization

1. Previously we reported that treatment of boar sperm with c-AMP-elevating drugs induced tyrosine phosphorylation of a triton-insoluble 93-kDa protein (p93). We have isolated p93 and identified it as a valosine-containing protein (VCP) by mass spectrometry and micro-sequencing. The c-AMP-elevating treatment did not alter VCP localization (sperm tail, posterior ring, distal equatorial segment, and post-acrosome), but induced tail tyrosine phosphorylation. In these sperm, VCP and pY colocalized in the connecting piece and posterior ring of the sperm head. Our data indicate that VCP is a substrate of c-AMP-activated boar sperm tyrosine kinase (1).

In a previous study, a series of monoclonal antibodies (Moab's) against boar capacitated sperm have been produced. One of these, 4B12, recognizes a surface-associated protein located in the acrosome portion of spermatozoa that became accessible to antibody after capacitation. In the biological experiments it was shown that Moab's significantly inhibited boar sperm-zona pellucida binding. The results documented the participation of the 4B12 protein in primary sperm-zona pellucida binding (2).

New monoclonal antibody (Hs-3) against human apolipoprotein J (ApoJ) was prepared. ApoJ is secondarily incorporated into the sperm membrane, as sperm travel through the male reproductive tract, and belongs to the sperm-coating proteins. The protein has been implicated in a variety of physiological processes including sperm maturation, lipid transport, and membrane remodelling (3).

We also participated in the study of a reverse effect of indomethacin on the immunosuppressive activity of boar seminal immunosuppressive fraction (ISF). We prepared monoclonal antibody ISF.7.E4, which similarly as indomethacin

reversed the inhibitory effect of ISF on mitogen-stimulated lymphocyte proliferation as well as on antibody production (4).

2. The effect of selected endocrine disruptors, xenoestrogens (bisphenol-A, nonylphenol, diethylstilbestrol) and phytoestrogens (genistein, resveratrol) on the integrated reproductive process as well as on individual reproductive organs and gametes was tested in the CD1 mice in a multigenerational study. Bisphenol-A, nonylphenol, and diethylstilbestrol had a negative influence on the number of born offspring of mice, on reproductive organs (Fig. 1b) and on the acrosome integrity of mouse spermatozoa (Fig. 2b). Contrary to that, treatment by phytoestrogens 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 (Fig. 2) (5,6,7).

Monoclonal antibodies against human intra-acrosomal proteins were used for detection of spermatids in ejaculates of men with azoospermia (ejaculate without spermatozoa). Our antibodies can bind to acrosomal proteins in round spermatids and prove spermatogenesis. The spermatids or spermatozoa obtained from testes by biopsy can be utilized in assisted reproduction. At present, one woman is pregnant in the Centre of Assisted Reproduction (8).

B. Cell growth control

Both thiol deprivation and thiol excess can induce apoptosis in lymphoma cells. Apoptosis induction by thiol deprivation is specifically related to the presence of a free SH group. However, apoptosis induction by thiol excess does not seem to be specifically related to the presence of a free SH group. It probably results from the excess of a reductant. Apoptotic control protein p53 does not seem to play a significant role in apoptosis induction either by thiol deprivation or by thiol excess (9).

In order to elucidate the mechanisms involved in apoptosis induction by iron deprivation, we studied the expression of p53 and the expression of selected p53-regulated genes in sensitive (human Raji, mouse 38C13) versus resistant (human HeLa, mouse EL4) cells under iron deprivation. The level of p53 mRNA decreased significantly under iron deprivation in sensitive cells, but it did not change in resistant cells. On the contrary, the level of the p53 protein under iron deprivation was slightly increased in sensitive cells while it was not changed in resistant cells. The activity of p53 was assessed by the expression of selected p53-regulated targets, i.e. $p21^{WAF1/CIP1}$ gene, *mdm2*, *bcl-2* and *bax*. We did not detect any relevant change in mRNA levels as well as in protein levels of these genes under iron deprivation with the exception of $p21^{WAF1/CIP1}$. We detected a significant increase in the level of p21 mRNA in both (sensitive and resistant) mouse cell lines tested; however, we did not find any change in both (sensitive and resistant) human cell lines. Moreover, the $p21^{WAF1/CIP1}$ protein was accumulated in mouse sensitive 38C13 cells under iron deprivation while all other cell lines tested, including human sensitive cell line Raji, did not show any accumulation of the $p21^{WAF1/CIP1}$ protein. It seems that the $p21^{WAF1/CIP1}$ mRNA, as well as protein accumulation, is not specifically coupled with apoptosis induction by iron deprivation and that it is rather cell line-specific. Taken together, we suggest that iron deprivation induces apoptosis independently of the p53 pathway (10).

Heat-shock protein 90 (Hsp90) is a molecular chaperone abundant in eukaryotic cells. However, its exact role is not completely understood yet. Employing an iron-binding assay and mass spectrometric analysis, we have identified human Hsp90 as an iron-binding protein in membrane protein preparations of human HeLa cells. Western blot analysis and confocal microscopy confirmed that a portion of cellular Hsp90 is associated with the plasma membrane,

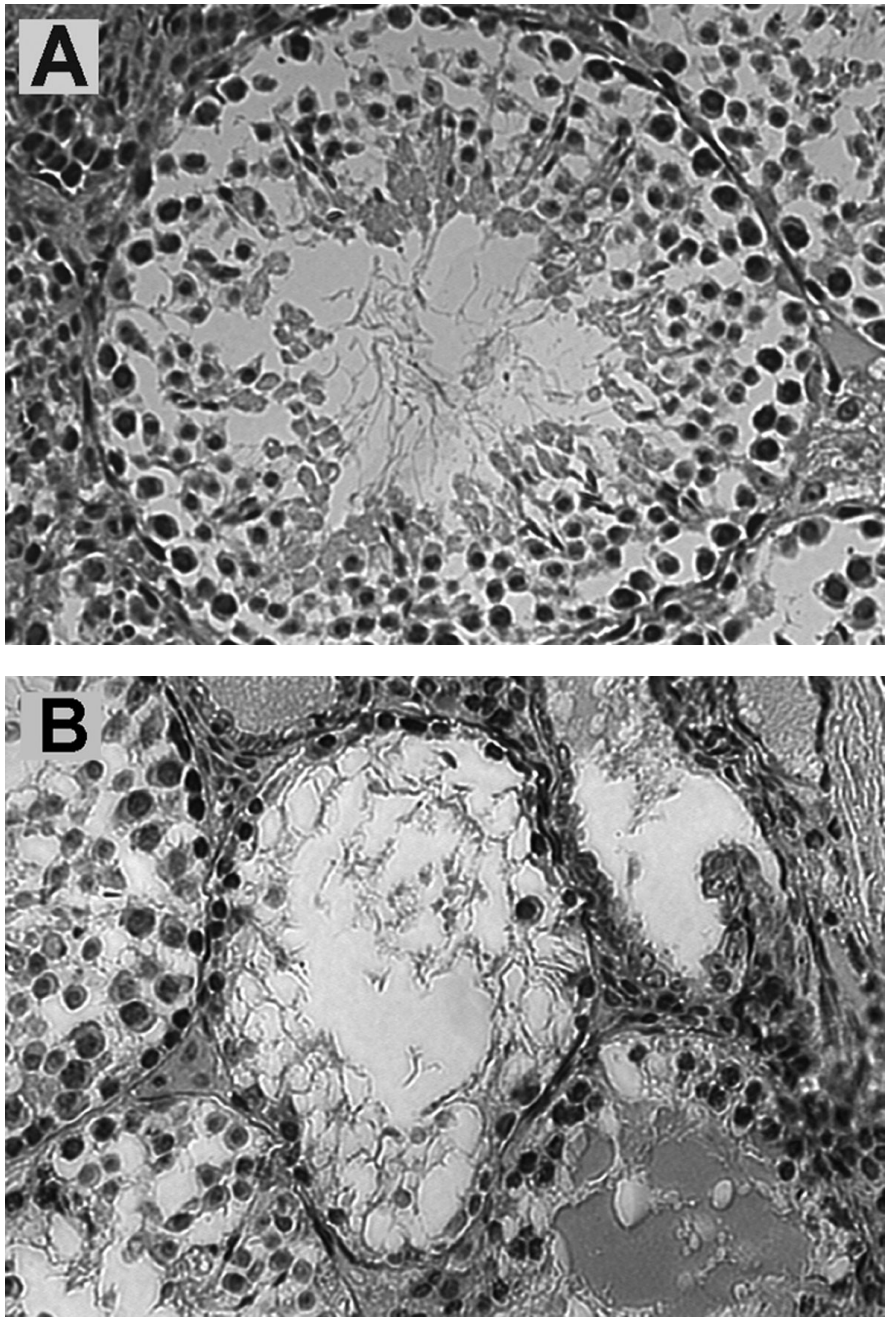

Fig. 1. The effect of bisphenol-A on spermatogenesis observed by the histochemical technique: control group **(A)** and 2 ng bisphenol-A in the second generation **(B)** (haematoxylin-eosin staining)

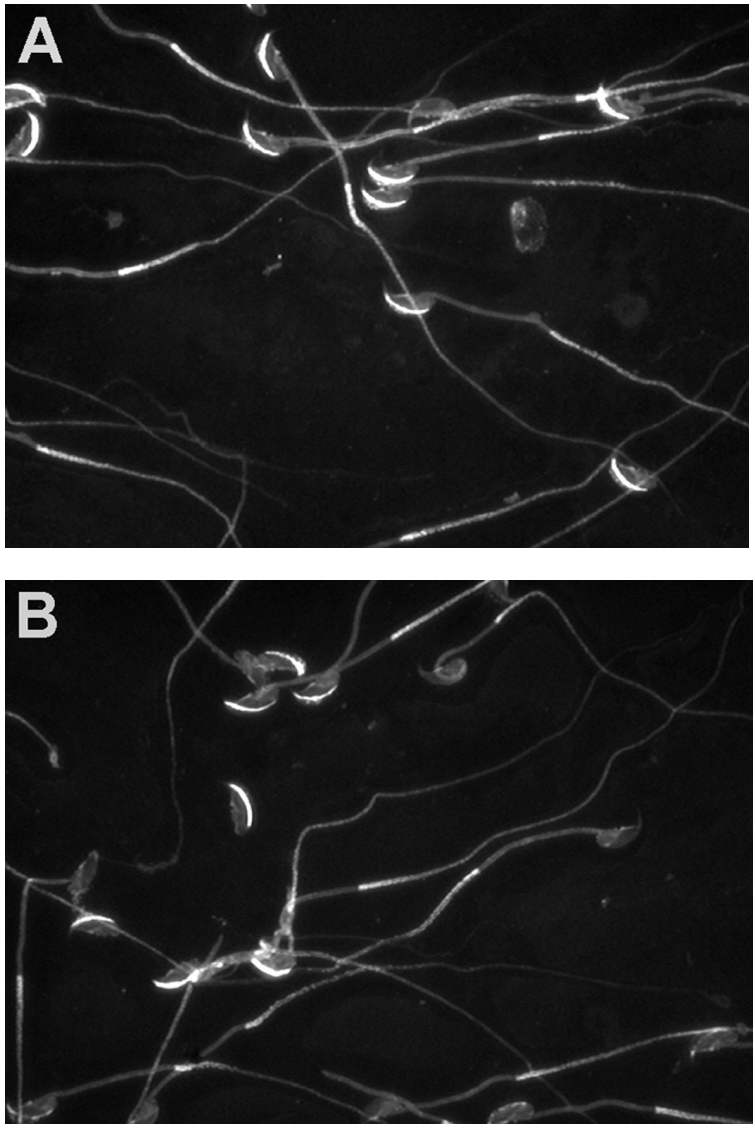

Fig. 2. Immunofluorescence staining of mouse sperm with MoAb Hs-14 in the control group **(A)** and in 2 ng bisphenol-A treatment in the second generation **(B)**

but it does not seem to be expressed on the cell surface. The iron-binding assay with purified human Hsp90 confirmed iron binding by Hsp90. Thus, we suggest that Hsp90 is an iron-binding protein associated with the plasma membrane (11).

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Biochemistry of Reproduction

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Undergraduate Students[1]*:* Martina Kublová (graduated 2002), Pavla Balínová (graduated 2003), Milan Pick (graduated 2003), Dagmar Hýblová, Eva Cibulková

Technicians: Magda Hošková (60%), Jitka Jelínková (80%)

[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 of the ovum and fusion of sperm and egg.

Seminal plasma proteins originate in seminal vesicles, prostate gland and bulbourethral gland. Some seminal plasma proteins are known to bind specifically to the sperm surface and interact with phosphorylcholine-containing components of the sperm membrane. The portion of proteins secreted by the prostate gland differs in various species. We have characterized the composition of boar prostate proteins. Fertilization involves a highly coordinated sequence of interactions between molecules on the surface and in the natural environment of both gametes. The lectin-like interactions play an important role in sperm surface protein interactions with saccharide chains of zona pellucida glycoproteins and partly in sperm binding in the oviductal reservoir.

Most boar seminal plasma proteins belong to spermadhesins and some of them contain the fibronectin type II domain. Under physiological conditions, almost all these proteins form aggregates of different relative molecular masses, compositions and binding properties. We have studied the mutual interactions of monomer forms of boar seminal plasma proteins that tended to aggregate and most probably contributed to rearrangement of the protein layer coating the sperm surface.

Proteinase inhibitors were found both in sperm extracts and in seminal plasma. Their biological role is to inactivate the prematurely released proteinase acrosin from occasionally damaged spermatozoa and thus to protect the male and the female genital tract against proteolytic degradation. We aimed to study the association of proteinase inhibitors with other components of boar seminal plasma.

We studied the relationship between the heparin-binding activity and the aggregation tendency of bull seminal plasma proteins and we were looking for the factors affecting aggregation and dissociation.

In parallel, biological properties of PSP proteins of boar seminal plasma were studied.

Results

We prepared a new affinity carrier with immobilized L-glyceryl phosphorylcholine, which re-

presents a cell membrane phospholipid interacting with seminal plasma proteins *in vivo*. The ligand was coupled directly to divinyl sulphone-activated Sepharose; the adsorbed proteins were specifically eluted with phosphorylcholine solution. The predominating component of the phosphorylcholine-binding fraction of bull proteins corresponded to the PDC-109 protein and that of boar proteins corresponded to the DQH sperm surface protein and spermadhesins of the AQN family. No phosphorylcholine-binding proteins were found in human seminal plasma (1).

Proteins of boar prostate gland separated by affinity chromatography on heparin-polyacrylamide were subjected to RP HPLC and their elution profiles were compared with boar seminal plasma. Interestingly, β-microseminoprotein was identified as the major protein of prostate secretion. PSP I and PSP II, the major proteins of the H^- fraction of boar seminal plasma, were found in boar prostate secretion in lower amounts. The major proteins of the H^+ fraction of boar seminal plasma (spermadhesins of the AQN, AWN families) were not detected in prostate secretion at all (2, 3).

Yeast mannan immobilized to divinyl sulphone-activated Sepharose was used for the isolation of mannan-binding proteins of bull seminal plasma, which were identified as RNAase dimer, PDC-109 and a protein homologous to BSP-30K. The isolated proteins showed a high binding activity to zona pellucida. Mannan inhibited the binding of bovine zona pellucida glycoproteins both to bull sperm and seminal plasma proteins; it might indicate the involvement of D-mannose-binding sites in the sperm-egg interaction (4).

Boar seminal plasma proteins adsorbed to immobilized spermadhesins corresponded to aggregated forms found in seminal plasma by size exclusion chromatography. Interactions between spermadhesins of the AQN and AWN families and the DQH protein, as well as between porcine seminal plasma proteins PSP I/PSP II forming a heterodimer were proved (5). These interactions participate in formation of protein aggregates in seminal plasma and probably also in the arrangement and remodelling of protein coating layers of sperm. Aggregation of seminal plasma proteins is probably an important phenomenon in the fertilization process. Proteinase inhibitors were found mainly in the gel chromatography fraction of the lowest relative molecular mass. Two serine proteinase inhibitors were isolated from boar seminal plasma (8 and 12 kDa): non-glycosylated sperm-associated acrosin inhibitor (SAAI-8 kDa) and glycosylated inhibitor of boar seminal plasma (12 kDa).

The SAAI-8 kDa proteinase inhibitor was proved to form a heterodimer with non-glycosylated AQN 1 spermadhesin. Glycosylation of the AQN 1 spermadhesin influences its behaviour, the non-glycosylated AQN 1 forms a heterodimer with SAAI-8 kDa; the glycosylated AQN 1 forms only a homooligomer. The 12 kDa inhibitor does not make a complex with AQN 1, but forms homodimers. We showed that not only spermadhesins, but also low molecular mass proteinase inhibitors participated in formation of associated complexes. We found two types of proteinase inhibitors that differ not only in their properties, but also in their presence in various protein complexes isolated from seminal plasma (6,7). The aggregated forms of boar seminal proteins have stable composition of individual molecular mass aggregates. In comparison with proteins of boar seminal plasma, the behaviour of bull seminal plasma proteins is different. Complicated polydisperse forms of main bull seminal plasma (BSP) proteins were found in seminal plasma. We succeeded in confirming that aggregation of BSP proteins depended on the components of its surrounding medium. The presence of D-fructose (as a component of seminal plasma) shifted relative molecular masses of protein-associated forms to lower values at size exclusion chromatography of whole seminal plasma and heparin-binding proteins. The higher was the heparin-binding activity of bull proteins, the lower was their tendency

to form aggregates, which might possibly play a role in capacitation (8).

The PSP I/PSP II heterodimer was proved as the immunosuppressive fraction (ISF) of boar seminal plasma. The protein part of the PSP II was found to bear the immunosupressive properties of the heterodimer (9). The ISF ability to inhibit mitogen-stimulated proliferation of splenocytes was reversed with a monoclonal antibody against ISF or by indomethacin, a non-steroid inhibitor of inflammation. ISF was shown to have a consistent effect on Concanavalin A-stimulated proliferation of splenocytes. It suppressed the Th1 cytokine (IL-2, IFN-gamma) production and stimulated the Th2 cytokine (IL-4, IL-6, IL-10) production. Besides, ISF significantly inhibited proliferation of B-lymphoma cells Ag 8 and X 63-IL-2 (10).

The work of the group was supported by the Grant Agency of the Czech Republic (Research Grants Nos. 303/02/0433, 303/02/P069, 303/01/0615, 524/01/0162), and by grants of the Ministry of Health of the Czech Republic (NJ/7463-3, NI/6883-3)

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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

Technicians: Pavel Lacina (part time), Veronika Lorincová (part time), Ivana Muricová (until December 2003), Olina Pertlíčková, Pavla Sojáková, Ivana Šebová, Kateřina Ševčíková, Markéta Tyšerová, Hana Vacková, 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 examinations of animals and monitoring of their health and genetic status. The facility is divided into an SPF unit and a monitored unit.

Mice required for grants are produced under SPF conditions. Production concentrates mainly onto gene knock-out and transgenic animals, mice from important congenic recombinat lines and mutants. Until now more than 40 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 two times a year. Strains are monitored genetically, each strain is controlled by transplantation every two years. The outcoming material used in 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 conditions. The SPF unit is open to other Institutes of the Academy of Sciences involved in biomedical research.

The monitored unit serves for routine short-time experiments. Animals in the monitored unit undergo routine examinations for their health status. A part of this unit is represented by 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 licenced for production of the animals. The monitored unit is used by researchers from the Institute of Molecular Genetics and some other Institutes of the Academy of Sciences in the Krč area. All researchers allowed to work in the monitored unit, as well as in the SPF unit, are trained and licenced for work with laboratory animals.

The staff members of the facility are trained and licenced to carry out breeding of and experimental work on laboratory animals according to the law for animal protection against cruelty No. 246/1992 Coll.

Journals Published by the Institute

Folia Biologica (Praha)

Folia Biologica (Praha), a journal of cellular and molecular biology, is published by the Institute of Molecular Genetics, Academy of Sciences of the Czech Republic (former Institute of Experimental Biology and Genetics, Czechoslovak Academy of Sciences).

The Journal traditionally focuses on gene expression and its regulation, biology and molecular biology of the eukaryotic cell, retrovirology and molecular genetics of the tumour cell, regulation of the immune response to malignant tumours, transplantation immunology, and immunogenetics.

The journal first appeared in 1954 and in 2004 is in its 50th volume. Published bimonthly, it contains reports of original research (both full-length and short communications) and occasional review articles. Monothematic issues dealing with the most recent "hot" topics are sometimes published, usually prepared upon the request of the editor. This peer-reviewed journal is indexed in several secondary services, including Current Contents/Life Sciences, Science Citation Index, Index Medicus and Excerpta Medica. It is supervised by an International Editorial Board consisting of 19 leading authorities from Europe and the United States.

Over the years the journal has published a number of excellent priority papers and has been very well received by the scientific community worldwide. The impact factor of the journal, according to the Science Citation Index, Journal Citation Report 2002, is 0.613. Abstracts of published papers are available on the World Wide Web (http://www.img.cas.cz/fb/). At this address, full-text papers are available with one year delay since 2000. Editorial office:

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Biologické listy

Biologické listy (Biology Letters), a national journal of molecular and cellular biology, is published by the Institute of Molecular Genetics, Academy of Sciences of the Czech Republic.

This journal is issued in the Czech and Slovak languages with abstracts in English. It is focused on biology, especially on the molecular and cellular biology, genetics, ecology, biochemistry, immunology and biotechnology.

The journal first appeared in 1935 and in 2004 is in its 69th volume. Published four times per year, it is focused on the presentation of systematic information on the progress and development of specific areas in different branches of biology. Its contents include current scientific data in the form of reviews as well as news articles, discussion contributions, and new book reviews, with a consideration of interdisciplinary relationship of these data within biological sciences. This peer-reviewed journal thus provides the main prerequisite for creation and maintenance of new Czech terminology, on which is based the education of classical and modern biology.

The journal should serve scientists and specialists in biology and related fields, secondary school and university teachers, undergraduate and postgraduate students, and, at the discussion level, all those who are interested in various problems of biology and biological research. More and more often, it serves as the first publication platform for the literary parts of student seminary works and doctorate theses. Editorial office:
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Lectures and Seminars

Thirty seminars were given at the Institute of Molecular Genetics by visiting scientists from other research organizations around the world within the years 2002 and 2003. Speakers came from eight foreign countries – Denmark, Germany, Italy, the Netherlands, Poland, Switzerland, 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.

Professor Bernard Fox, Ph.D. (Earle A. Chiles Research Institute and Oregon Cancer Center, Portland): How Do T Cells Mediate Tumor Regression? January 14, 2002.

Professor David Jones (Phillipps University, Marburg): Imaging in Space. March 25, 2002.

Professor Paula Pitha-Rowe, Ph.D. (Johns Hopkins Sch. Med., Baltimore): Interferon Regulatory Factors: Their Role in Innate Immunity and Viral Mimicry. March 28, 2002.

Dr. Peter Démant (The Netherlands Cancer Institute, Amsterdam): Genetic Dissection of Complex Diseases in Mice: Cancer Susceptibility Genes. April 26, 2002.

Professor Rolf Hilgenfeld (Institute of Molecular Biotechnology, Jena): Bacterial Virulence Factors and Protein Folding. May 14, 2002.

Professor Keith E. Latham (Temple University School of Medicine, Philadelphia): Epigenetic Modifications of Embryonic Genome. June 5, 2002.

Professor Knud H. Nierhaus (Max-Planck-Institute for Molecular Genetics, Berlin): The Ribosomal E-Site Story. July 10, 2002.

Professor Abraham Karpas (University of Cambridge, Cambridge): A Human Myeloma Cell Line Suitable for the Generation of Human Monoclonal Antibodies. July 10, 2002.

Professor Roger S. Goody, Ph. D. (Max-Planck-Institute of Physiology, Dortmund): The Structural Basis of Post-translational Prenylation of Rab Proteins, the Central Regulators of Vesicular Transport. August 5, 2002.

Dr. Maria Ruzzene (University of Padova, Padova): Implication of Protein Kinase CK2 in Apoptosis. September 12, 2002.

Professor Josef Jiřičný (Institute of Medical Radiobiology, University of Zürich, Zürich): The Role of Mismatch Repair Proteins in Cell Cycle Arrest and Colon Cancer. September 18, 2002.

Thomas R. Cech (Howard Hughes Medical Institute, Chevy Chase, Maryland): Life at the End of the Chromosome: Telomeres and Telomerase. October 18, 2002.

Alasdair Gilfillan, (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda): Studies on the Role(s) of PI-3 Kinase in the FcεRI and FcγRI Dependent Degranulation of Human Mast Cells. November 6, 2002.

Professor Harald zur Hausen, M.D. (Deutsches Krebsforschungscentrum, Heidelberg): Vaccines for Cancer Prevention and Therapy. November 7, 2002.

Dr. Mariola Wojtczak (Institute of Animal Reproduction and Food Research of Polish Academy of Science, Olsztyn): Proteinase Inhibitors in Cyprinids Seminal Plasma. December 5, 2002.

Dr. Mariola Kotlowska (Institute of Animal Reproduction and Food Research of Polish Academy of Science, Olsztyn): Proteolytic Activity in the Seminal Plasma of Turkey Normal and Yellow Semen During Reproductive Season. December 5, 2002.

Dr. Qunyan Yu (Dana Farber Cancer Institute, Boston): G1 Cyclins in Mammary Tumorigenesis. March 27, 2003.

Professor Ulf Dittmer (Institute of Virology, University of Essen, Essen): Vaccination and Immunotherapy against Retroviruses: Lessons from the Friend Murine Leukemia Virus Model. May 15, 2003.

Professor Ludvík R. Donner, M.D. (Scott &White Memorial Hospital & Clinic, The Texas A&M University College of Medicine, Temple): Cytogenetics of Tumours of Soft Tissues. Classification, Diagnostic, and Molecular Biology Implications. May 29, 2003.

Professor Lorenzo A. Pinna (University of Padova, Padova): The Raison D'Etre of Constitutively Active Protein Kinases: the Lesson of CK2 („Casein Kinase-2"). May 30, 2003.

Professor Roberto P. Revoltella, M.D., Ph.D. (Istituto di Mutagenesi e Differenziamento Reparto di Immunobiologia e Differenziazione Cellulare, Pisa): From Stem Cells to Epithelial Cells. June 5, 2003.

Professor Richard D. Cummings, Ph.D. (University of Oklahoma Health Sciences Center, Oklahoma City): Galectin Interactions with Leukocytes: Signaling and Ligand Recognition. June 9, 2003.

Professor Josef Jiřičný (Institute of Medical Radiobiology, University of Zürich, Zürich): Repair of G/T Mispairs Arising through Deamination of 5-methylcytosine. June 25, 2003.

Adam Pavlíček, Ph.D. (Genetic Information Research Institute, Mountain View): Chromosomal Territories and Gene Expression: Is the Specificity of Tissue Expression Really Determined by Promoters? *In silico* Approach to the Human Genome. September 24, 2003.

Dr. Robert Belshaw (Imperial College at Silwood Park): The Evolution of Human Endogenous Retroviruses. September 24, 2003.

Professor Jiří Bartek (Institute of Cancer Biology, Danish Cancer Society, Copenhagen): Cell Cycle and DNA Damage Checkpoints: Mechanisms and Disease-predisposing Defects. November 24, 2003.

Dr. Konrad J. Böhm (Institute of Molecular Biotechnology, Jena): Kinesin's Work in Cell-free Environment for Future Nanoactuatoric Developments. September 26, 2003.

Dr. Jiří Lukáš (Institute of Cancer Biology, Danish Cancer Society, Copenhagen): Molecular Control over the Cell Cycle Transitions: A Key to Avoid Cancer-predisposing Genetic Instability. November 24, 2003.

Dr. Zbyněk Bozděch, Ph.D. (University of California, San Francisco): The Transcriptome of the Intraerythrocytic Developmental Cycle of *Plasmodium falciparum*. December 15, 2003.

Dr. Daniel Zicha (Cancer Research UK London Research Institute, London): Motility, Chemotaxis and Gene Expression Microarrays in Metastasising Sarcoma Cells. December 15, 2003.