50 Years of Czech and Slovak Yeast Research

(Period 2000-2009)

Bratislava, 2010
Commission on Yeasts, Czechoslovak Microbiological Society

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ISBN:978-80-89257-20-1
Bratislava 2010
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Chairpersons of the Czechoslovak Commission on Yeasts

RNDr. Anna Kocková-Kratochvílová, DrSc. (1964-1983)


RNDr. Peter Biely, DrSc. (1993 – 2002)

Assoc. Prof. Ing. Vladimír Farkaš, DrSc. (2002-present)
PREFACE

The booklet that you are holding in your hands has been issued to commemorate the 50th anniversary of the existence of the Commission on Yeasts of the Czechoslovak Microbiological Society. With this publication, we are continuing the tradition of periodic decennial almanacs issued by the Commission on Yeasts of the Czechoslovak Microbiological Society with the intention to give a brief and comprehensive view on the activities and achievements of Czech and Slovak yeast researchers in the last decade. The first three books of this series [1-3] were edited by the late Dr. Anna Kocková-Kratochvílová (passed away in 1992) who will be remembered as the founder of the Czechoslovak Commission on Yeasts, creator of the Czechoslovak Yeast Collection and organizer of activities of yeast researchers both at home and all over the world. Dr. Kocková-Kratochvílová, together with Dr. Erich Minárik (passed away in 2007), had a lion’s share in the creation of the international organization, which is now recognized as the International Commission on Yeasts (ICY) [4,5]. In 1981 ICY has become part of the Mycology Division of the International Union of Microbiological Societies. The birth of ICY took place during the 2nd International Symposium on Yeasts held in Bratislava, capital of Slovakia, in July 1966.

The ten years that have elapsed since the appearance of our last almanac [6] have been marked by unprecedented expansion of yeast research into various areas of biological sciences. The yeast cells have been introduced as models of eukaryotic cells into diverse fields of biological research, such as cell cycle, apoptosis, congenital disorders of glycosylation, cancer or neurodegenerative diseases. The importance and contribution of yeast studies to the development of science in general have been acknowledged by three Nobel prizes awarded to yeast scientists in the last decade (Lee Hartwell, Paul Nurse and Timothy Hunt in 2001, Roger Kornberg in 2006, and lastly Jack W. Szostak together with Elisabeth H. Blackburn and Carol W. Greider in 2009). The increasing amount and importance of studies on yeasts have been reflected also in founding of a new prestigious scientific journal dealing specifically with yeasts – the FEMS Yeast Research.

For the Czech and Slovak community of yeast researchers, the last ten years have been marked first by association and later the full membership of our countries in the European Union. This act has opened the way to our equal partnership in the European research programs and in multilateral international research projects. Some of Czech and Slovak yeast research groups were successful in gaining grants from the European Framework Programs and from the prestigious Howard Hughes Medical Institute. The funds obtained through the international grants helped to improve the research infrastructure and in many cases to realize more complex and financially demanding research plans. The quality of research has markedly improved and many works of Czech and Slovak yeast researchers have found their way into highly impacted scientific journals.

The annual yeast conferences and their romantic venue, the Smolenice Castle, are part of the tradition that has been piously respected by our Commission on Yeasts for the past 50 years. Nevertheless, in spite of traditions, the character of our conferences considerably changed during the last 10 years. From the small, almost private “family” gatherings where communication was held in Czech and Slovak languages, they have evolved into scientific events of international character and importance. In 2003, English was established as the official conference language. As a rule, distinguished yeast researchers from home and
abroad have been invited to deliver plenary lectures and the opening talks devoted to the memory of Dr. Kocková-Kratochvílová. The website of the Czechoslovak Commission on Yeasts (http://www.chem.sk/yeast/) has been placed on the internet enabling online registration and information of the yeast research community. For the young yeast researchers (the so-called yeasties), our annual conferences represent the first opportunity to present their results in front of an international audience.

The popularity of our annual conferences has markedly increased not only among Czech and Slovak members but also among the yeast researchers from the neighboring countries. The efforts of the organizers to increase the scientific and social standards of the annual conferences have been to a great deal supported by the sponsors having some relations to yeast research, such as the brewing industry, local wineries and chemical suppliers. Some conferences got the financial support from the International Visegrad Fund (IVF) intended to foster the regional cooperation between Slovakia, Czech Republic, Poland and Hungary.

We would like to express the hope that future years will be marked by further development of yeast science in our countries. May the annual conferences of the Czech and Slovak Commission on Yeasts at the Smolenice castle be the place where the new ideas are born and disseminated, the place to where the yeast researchers will always gladly return.

Assoc. Prof. Vladimír Farkaš, DrSc., Chairman of the Czech and Slovak Commission on Yeasts (2002 – present)
RNDr. Peter Biely, DrSc., former Chairman of the Czech and Slovak Commission on Yeasts (1993 – 2002)

References

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Research field, aims and directions

Biochemistry of utilization of plant polysaccharides by yeasts and other microorganisms; microbial degradation of plant cell walls; search for novel glycoside hydrolases and carbohydrate esterases; production, mode of action and biotechnological potential of these enzymes.

Individual topics pursued in the group and principal results

1. Biochemical characterization of plant polysaccharides-degrading enzyme systems produced by microorganisms including yeasts. Study of enzyme components, their cellular localization, regulation of their synthesis and nature of inducers.

2. Diversity of substrate recognition by microbial endoxylanases. Characterization of endoxylanases belonging to different glycoside hydrolase families, their substrate specificity, tolerance of xylan side chains and substrate recognition sites.


4. Xylanolytic enzyme system of the xylose-fermenting yeast *Pichia stipitis* specialized on utilization of hardwood glucuronoxylan. Natural habitat of this yeast is digestive gut of beetles eating hardwood. The yeast lives in a consortium of microorganisms and its secreted enzyme machinery shows a low efficiency of xylan degradation. Exceptions are some glucuronoxylan debranching enzymes. The yeast appears to be a promising source of novel enzymes degrading plant hemicelluloses.

5. New family of glucuronoxylan-debranching enzyme produced by *Pichia stipitis* has been discovered. Based on its gene isolation a new family of GH has been introduced (GH115). Research pursued in this direction concerns catalytic properties of the enzyme, the stereochemistry of its hydrolytic mechanism and determination of its three-dimensional structure.
List of relevant publications (2000-2009)


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Research field, aims and directions

• Resistance, virulence factors and modulation of their expression in clinically important Candida yeasts (C. albicans, C. dubliniensis, C. glabrata, C. parapsilosis).
• Biochemical and immunological characterization of cell wall proteins from Candida spp. participating in fungal-host interactions.
• Biofilms - the new medical problem associated with resistance to host defense system as well as to antimicrobial agents.

Individual topics pursued in the Laboratory and principal results

Resistance mechanisms, virulence factors, biofilm formation

Our research is focused on monitoring resistance mechanisms to antifungal agents and searching for new possible targets for antifungal drugs in the treatment of diseases caused by pathogenic yeasts C. albicans a C. dubliniensis. In this study, a collection of resistant clinical isolates was involved. The main interest was concerned on the expression of efflux transporters (Cdr1p, Cdr2p, Mdr1p) and changes in the expression of genes involved in ergosterol pathway (ERG1, ERG3, ERG7, ERG9, ERG11 a ERG25). Research also includes comparison of the occurrence of key resistance mechanisms in C albicans and C. dubliniensis during biofilm formation. Another important topic is aimed at interaction of yeasts with biotic and abiotic surfaces during adherence, the critical phase of biofilm development. The last part concerns the immunomodulatory effect of the Candida antigen - CR3-RP (complement receptor 3-related protein) that is supposed to be a “mimicry” protein of the mammalian CR3 (CD11b/CD18).

List of relevant publications (2000-2009)


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Research field, aims and directions

Studies on yeast cell wall polysaccharides, α-D-mannans and β-D-glucans concerning (i) structure of cell wall polysaccharides (5,44,46); (ii) water soluble derivatives of β-D-glucan and their protective function as antioxidants, antimutagens, and antigenotoxic agents (1,2,4,6-11,13-17,19-21,23-30,32-36,45,50-53,57); (iii) glycoconjugates and their immunobiological properties (3,12,18,22,31,37-43,47-49,54-56,58).

Structure of cell wall polysaccharides
Diseases caused by the yeast of Candida genus are a serious clinical problem. Despite this fact, there is as yet no effective prevention against these opportunistic pathogens. Although C. albicans is the major cause of the mycoses (67%), the number of the multiresistant non-albicans isolates increases. C. dubliniensis, which was described only recently as serious human pathogen, belongs to the group of these resistant isolates. The cell wall mannan of Candida cells participates in an initial contact with its host and subsequently with the host defense mechanisms. Because of the complexity of this homopolymer antigen it was necessary to identify a subunit of the mannan C. dubliniensis that is most effectively recognized by the immune system and thus influences the specificity of the induced antibody response. The detailed structure of surface mannan of C. dubliniensis was firstly described using NMR analysis.

Derivatives of β-D-glucan and their protective function as antioxidants, antimutagens, and antigenotoxic agents
Glucan-based preparations obtained from industrial biotechnologically important fungal strains can serve as broad spectrum immunomodulators. Because the source of these valuable polysaccharides is quite common, abundant and inexpensive, more attention should be paid to their utilization in polysaccharide biotechnology. Besides, fungal (1→3)-β-D-glucans have the ability to stimulate the immune system and are classified as biological response modifiers (BRMs). They are water-insoluble, therefore ultrasonic treatment and subsequent chemical derivatization (carboxymethylation and sulfoethylation) is necessary for preparation of water-soluble derivatives. It has been found that the derivatives prepared from the cell wall of S. cerevisiae reveal high mitogenic and comitogenic activities, as well as radioprotective and antimutagenic effects. Also, the antimutagenic effects of glucomannan isolated from C. utilis against cyclophosphamide in mice were confirmed. Both i.p. and p.o. administration of glucomannan prior to cyclophosphamide injection decreased significantly the frequencies of micronuclei in bone marrow compared to the control groups. Glucomannan could be of great
prospective value as a natural protective agent against the adverse effects of cyclophosphamide.

**Glycoconjugates and their immunobiological properties**

The cell surface components such as the polysaccharides are dominant cell-wall structures recognized by the immune system. Fungal polysaccharides represent pathogen-associated molecular patterns, conserved microbial structures stimulating innate and adaptive immunities via Toll-like receptors and other pattern-recognition receptors expressed by several immunocompetent cells such as neutrophils, macrophages, or dendritic cells. This recognition plays a significant role in host defence and presents specific opportunities for active immunomodulation of the host immune response. The enhancement of antifungal immunity requires T-cell-dependent B-cell activation. Polysaccharides as weak immunogens are considered to give an immune response independent of T lymphocytes. Direct conjugation of yeast mannans with protein is an effective route of antifungal vaccine preparation. Immunodiffusion experiments revealed that conjugation did not affect the immunospecificity of the antigen (mannan) epitope. The conjugation of polysaccharide antigens with protein carrier molecules considerably enhances their immunogenicity towards T-cell dependency. Our experimental results in animal model indicate effective immunomodulating activities of yeast cell wall polysaccharide structures and provide encouraging findings for their use in subcellular vaccine development. The synthesized prototype mannan conjugates outline a new possible way of vaccine preparation against fungal infections that have recently experienced an enhanced dissemination.

**List of relevant publications (2000-2009)**


49. Paulovičová E., Bystrický S., Machová E., Bujdáková H.: Immune


Fig.: Direct immunofluorescence detection of localization and intensity of dimannoside and/or pentamannoside expression in different *C. albicans* CCY 29-3-162 morphological forms, from single-cell budding yeast form (A) to a multicellular filamentous form (B). Photo E. Paulovičová
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Research field, aims and directions

The budding yeast *Saccharomyces cerevisiae* has already proven to be a powerful model system for DNA repair studies on both cellular and molecular levels. Over the past ten years, our group has mainly focused on the mechanisms of DNA double-strand break (DSB) repair in this organism after treatment with oxidizing and cross-linking agents, as well as on other processes that contribute to chromosomal instability. Moreover, we have been using yeast model system to understand the underlying mechanisms of human disease that is associated with defective DSB repair.

Individual topics pursued in the Yeast Group of the Laboratory of Molecular Genetics and principal results

*RecA-like proteins in yeast and their role in the repair of DNA double-strand breaks*

RecA protein, the main *Escherichia coli* recombinase, complemented the DSB repair defect of the *S. cerevisiae* rad52 mutant cells suggesting that Rad52 may have some biochemical activity common with RecA (5, 9).

*Possible role of the Snm1/Pso2 protein in repair of DNA double-strand breaks in S. cerevisiae*

*The S. cerevisiae* Pso2 protein operates in the repair of the interstrand cross-links (ICLs)-associated DSBs. To address the question whether the function of Pso2 in the repair of these DSBs is mediated through protein-protein interactions, we conducted a comprehensive two-hybrid screen examining a possibility of interaction of Pso2 with all known DSB repair factors. We showed that Pso2 associates with none of the DSB repair proteins, suggesting that this protein very likely does not act in the repair of ICL-associated DSB via crosstalk with DSB repair machinery. Instead, its function in this process seems to be rather individual (12, 13).

*Repair of DNA lesions induced by oxidative stress in S. cerevisiae*

We demonstrated that repair of bleomycin (BLM)-induced DSB requires both DSB repair pathways, homologous recombination (HR) and non-homologous end-joining (NHEJ) (4, 7, 10, 14). However, HR is much more important for repair of BLM-induced DSBs than NHEJ. Cytotoxic effects of two other oxidizing agents studied, namely hydrogen peroxide and menadione, are caused mainly by other types of DNA damage than DSB (11).
Toxicity and mutagenicity of selenium compounds in S. cerevisiae

Sodium selenite (SeL), selenomethionine (SeM) and Se-methylselenocysteine (SeMC) were tested for toxic and mutagenic effects, as well as DNA double-strand breakage potencies in S. cerevisiae. Only SeL manifested any significant toxic effects, which were accompanied by pro-mutagenic effects in the stationary phase of growth. The toxic and mutagenic effects of SeL are likely associated with the ability of this compound to generate DSBs (16). Furthermore, we reported that the Rad52 protein is indispensable for repairing SeL-induced DSB suggesting the fundamental role of HR in this process in the yeast S. cerevisiae (17).

Further in vivo characterization of the mutant and polymorphic DNA ligase IV proteins found in LIG4 patient

The main pathway for the repair of DSB in mammals is NHEJ, in which the DNA ligase IV/XRCC4/XLF complex (LXX) is the key player (15). Until now, twelve LIG4 patients have been reported, each with at least one hypomorphic mutation in the DNA ligase IV gene (LIG4) and one with two linked polymorphisms. Some mutant and polymorphic changes were already characterized in vitro, although in vivo data are rather limited due to a lack for sensitive in vivo assays in mammals. We have been further characterizing the impact of the mutant and/or polymorphic changes on LXX function in vivo. This is being achieved by heterologous expression of the mutant and polymorphic LXX in S. cerevisiae and by monitoring the efficiency and accuracy of DSB rejoining in well-defined systems. Moreover, the impact of mutant and polymorphic LXX on chromosomal instability is being examined in this project.

List of relevant publications (2000-2009)

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Research field, aims and directions of the group

The group consists of teachers/researchers and PhD students belonging to the Department of Biochemical Technology FCHPT STU. The group is oriented on regulation of microbial overproduction of biologically active and industrially attractive lipid structures, pigments and polysaccharides, study of molecular and biochemical mechanisms controlling the biosynthesis of these compounds, characterization of their properties and their applications in food, pharmaceutical, veterinary and medical fields. The work of the group is also focused on scale-up that is important for transfer of results from basic research to applied field. The group cooperates with several research teams in Slovakia, such as the Institute of Chemistry, Slovak Academy of Sciences in Bratislava (Dr. E. Breierová, PhD – study of pigment and glycoprotein production), Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences in Bratislava (Dr. I. Hapala, PhD. – study of lipid biogenesis), Department of Physical Chemistry FCHPT STU Bratislava (Assoc. Prof. P. Rapta, DSc. – study of antioxidant and radical scavenging properties of yeast metabolites). The main collaboration of the group with foreign research teams is closely related to Faculty of Chemistry, VUT Brno (Assoc. Prof. I. Márová, PhD. – biotechnological regulation of pigment production).

Individual topics pursued in the Laboratory and principal results

Effect of stress on lipid metabolism in yeasts

Cells possess adaptation mechanisms for elimination of undesirable environmental factors. Membrane is the site of the primary contact of the cell with its environment. Phospholipids as the basic structural elements of membranes restructure fatty acid composition according to environmental alterations. Neutral lipids did not show such intensive changes in fatty acid composition as their polar counterparts. However, alterations in fatty acid profile in individual lipid classes depended on yeast strain sensitivity to stress conditions. For example, while NaCl induced synthesis of linoleic and linolenic acid in Sporabolomyces roseus and
*Rhodotorula rubra*, reduced conversion of 18:1 to 18:2 was observed in *Saccharomyces cerevisiae* stressed by NaCl. Salt also enhanced linoleic acid content in membrane lipids of *Cystofilobasidium capitatum*. Zn²⁺ stimulated the synthesis of monounsaturated fatty acids (palmitoleic and oleic acids) by activated Δ⁹ desaturase and inhibited the transformation of oleic acid to linoleic acid. Ni²⁺ ions suppressed the biosynthesis of linolenic acid from linoleic acid more than Zn²⁺ ions. Copper enhanced linoleic acid formation and selenium increased the levels of both linoleic and linolenic acids in phosphatidylcholine and phosphatidylethanolamine in red yeasts. It is assumed that activities of membrane-bound Δ⁹, Δ¹² and Δ¹⁵ desaturases regulating the synthesis of oleic, linoleic and linolenic acids and their subsequent incorporation to main membrane lipids reflect the adaptation changes of yeasts and are responsible for maintenance of vital membrane functions of stressed yeasts.

**Carotenoid pigment production**

*Rhodotorula* and *Sporobolomyces* synthesized beta-carotene as the main pigment together with torulene and torularhodine as minor carotenoids. *Phaffia* strains accumulated astaxanthin as a principal carotenoid. Overall yield of carotenoids is directly related to the total biomass yield, thus, to keep both high growth rates and high carbon flow efficiency to carotenoids by optimal cultivation conditions is essential in order to achieve the maximal pigment productivity. Factors such as carbon and nitrogen sources, minerals, vitamins, pH, aeration, temperature, light and stress showed a major influence on cell growth and yield of carotenoids. Major attention has been focused to the study of heavy metals on pigment biosynthesis by red yeasts. From these studies it is evident that zinc maximally stimulated accumulation of both β-carotene and torulene in yeasts while copper enhanced formation of torularhodin. On the other hand, production of carotenoid pigments was suppressed by selenium. Combination of various stress factors (e.g. salt, metals, hydrogen peroxide) resulted in activated carotenoid biosynthesis. Changes in pigment production caused by heavy metals might be explained by two hypotheses: a) possible activation/inhibition of specific enzymes involved in carotenoid biosynthesis, and b) the presence of heavy metals results in the formation of various active oxygen radicals that, in a turn, induces generation of protective carotenoid metabolites counteracting the deleterious action of free radicals. Optimized conditions in laboratory were verified in semi-pilot-scale experiments and yielded up to 35 mg β-carotene/L and 110 mg astaxanthin/L, respectively. Thus, red yeast biomass enriched with both pigments and essential trace elements (e.g. selenium) might be used for preparation of a new type of antioxidant formula that could be directly employed for various diets.

**Production of extracellular glycoproteins**

Induced stress triggers several mechanisms to maintain proper viability of red yeasts. Biosynthesis of compatible solute molecules outside of the cell (e.g. exoglycoproteins) is an important adaptable response of yeasts to heavy metal presence. Exoglycoproteins formed by *Pichia anomala* and *Candida maltosa* are characterized by high metal binding capacity and elevated levels of
polysaccharides. Copper or selenium stimulated accumulation of xylose in exoglycoproteins. Selenium increased the levels of proteins in exoglycoproteins in yeasts. Extracellular glycoproteins effectively captured heavy metals from media (e.g. up to 80% of zinc) and reduced their penetration into the cells. Thus, yeast extracellular glycoproteins with the capacity to capture heavy metals from the environment could be applied in bioremediation and detoxification processes.

**Antioxidant and radical scavenging properties of yeast extracts**

One effective method of measuring the ability of yeast carotenoids to quench free radicals is based on EPR spectroscopy with the use of spin trapping. The thermal decomposition of K₂S₂O₈ in H₂O/DMSO solutions at 333 K was used as a powerful source of both oxygen- and carbon-centered reactive radicals. DMPO (5,5-dimethylpyrroline N-oxide) was used as spin trap. The total antioxidant properties were additionally investigated spectrophotometrically using ABTS-based method and Ferric Reducing Ability of Plasma (FRAP) method. Antioxidants present in fibrillar part of cell walls showed much higher ability to scavenge free radicals than those from cells. Zn²⁺ ions and hydrogen peroxide induced changes in yeast leading to more efficient scavenging and antioxidant capacities as compared with Cu²⁺ and Ni²⁺ ions. Completely different reactive radicals were detected when yeasts were stressed by cupric ions in comparison to yeasts stressed with Ni²⁺ and Zn²⁺ ions. These results contain important information about the influence of metal ions on yeasts, the chemistry of oxidative processes and the action of antioxidants.

Antioxidant and radical scavenging properties of cell wall glycoproteins from yeasts growing in various morphological forms were also tested. The ability of yeast glycoproteins to quench both oxygen- and carbon-centered reactive radicals was evaluated by the thermally initiated decomposition of K₂S₂O₈ at 333 K coupled with EPR spin trapping (5,5-dimethylpyrroline N-oxide [DMPO] was employed as a spin trap). Glycoproteins isolated from “yeast-like” form of *C. albicans* eliminated reactive •OH radicals the most effectively. Similarly, ABTS test (suitable for proton-donating measurements) showed the best antioxidant properties for glycoproteins isolated from “yeast-like“ form of *C. albicans*. It is interesting that glycoproteins isolated from cell walls of *R. glutinis* and *P. pastoris* displayed the best antioxidant capacities when strains were grown in yeast budding form.

**List of relevant publications (2000-2009)**


Giant colony of *Rhodotorula* sp.  
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Research field, aims and projects

Research projects in the Laboratory are concerned with the structure, function and biosynthesis of yeast cell walls, *Saccharomyces cerevisiae* and *Cryptococcus neoformans* being used as model organisms.

Individual topics pursued in the Laboratory and principal results

*Effects of osmotic shock on the yeast cell wall*

Sudden increase of osmolarity of the growth medium causes temporary arrest of growth of yeast cells. After about 2 h in hypertonic medium, the yeast resumes growth at the original rate. The osmotic shock causes temporal restructuring of the yeast cell wall and disaggregation of the cytoskeleton. The restructuring of the cell wall is manifested as localized unmasking of the skeletal cell wall polysaccharides \(\beta\)-glucan and chitin. In the presence of Calcofluor white, the unmasked areas of the cell wall are revealed as fluorescent patches on the cell surface. After an adaptation period of about 2 h, the cell wall surface and the cytoskeleton morphology return back to normal [1].

The osmotic shock has also an effect on the yeast plasma membrane which becomes permeable to small molecules, thereby enabling the measurement of intracellular enzyme activities *in situ* [2]. Attention has been devoted to analytical methods employed in determining the cell wall composition and structure [3] and in cell wall functional genomics [4].

*Metabolic stability of the cell wall and capsule in Cryptococcus neoformans*

A number of ecto-enzymes of the glycanase type and located at the cell periphery (in the cell wall and/or in the capsule) of the yeast *C. neoformans* were detected. In spite of their presence, the yeast is unable to use its own capsular polysaccharides as an energy source [6]. An acidic endo-\(\beta\)-1,3-glucanase whose synthesis is derepressed at the end of logarithmic phase of growth causes dissolution of the cell wall resulting in cell death. As a unique rescue mechanism, the cells synthesize *de novo* secondary cell walls underneath the old ones [10]. This strategy is entirely different from the cell wall integrity mechanism operating in *Saccharomyces cerevisiae* as a response to cell wall stress. Whereas in *S. cerevisiae* the damaged or stressed cell walls are being rescued by activation of the synthesis of chitin, mannan and their increased cross-linking, in *C. neoformans* the weakened cell walls are being replaced by newly formed complete cell walls. The new, secondary
cell walls have increased contents of skeletal cell wall polysaccharides β-glucan and chitin [11].

**Formation of inter-polymeric linkages in the cell wall**

The yeast cell wall is a composite structure containing polysaccharides and proteins mutually linked by covalent bonds. The linkages between the individual polymers are most probably formed *in situ*, in the cell wall. Mutual bonding of the polymers represents the final phase in cell wall formation. One of the enzymes that could possibly catalyze the formation of ester type linkages between proteins and polysaccharides is transglutaminase [7, 9]. Other types of cross-linking enzymes are transglycosylases catalyzing the formation of intermolecular linkages between polysaccharide molecules in the yeast cell wall. We have developed a sensitive fluorescent assay for determination of activity of Crh1p and Crh2p - transglycosylases located in the cell walls and mediating the formation of covalent linkages between chitin and β-(1,6)-glucan. Cell walls isolated from the wild strain and from mutants crh1 and crh2 were shown to incorporate sulforhodamine-labelled β-1,3-linked and β-1,6-linked glucooligosaccharides *in vivo* and *in vitro*. Digestion of the labeled cell walls with specific hydrolases proved that the labeled oligosaccharides were linked to chitin [10]. Crh1 and Crh2 were cloned and heterologously expressed in *Pichia*. Using the fluorescent assay, their biochemical properties were determined. Both Crh1p and Crh2p use carboxymethyl chitin as the exclusive donor substrate and β-1,3-linked labeled glucooligosaccharides as the acceptors. In addition, Crh2p can use also β-1,6-linked glucooligosaccharides as the acceptors [12].

**List of relevant publications (2000-2009)**


**Fig.:** (A), Early stationary-phase culture of *Cryptococcus neoformans*. The remnants of the primary cell walls are attached as caps (arrows) to the cell surfaces; (B), ELM of an ultrathin section of a *C. neoformans* cell with formed secondary cell wall (arrows). Photo V. Farkaš, M. Sipiczki.
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Research field, aims and directions  

**Coupling of transcription and pre-mRNA processing in the regulation of gene expression**

We would like to contribute to the understanding of how transcription initiation, RNApolII elongation, and splicing are coordinately regulated during gene expression. We use *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* to study evolutionarily conserved regulatory factors of the synthesis and maturation phases of transcript production.

Individual topics pursued in the Laboratory and principal results  

**SNW1/Prp45/SKIP project**

The gene for the co-regulator SNW/SKIP has been identified and characterized by our group in *Dictyostelium discoideum* and *Schizosaccharomyces pombe*. We documented the interaction between the *S. pombe* SNW/SKIP protein and an essential spliceosomal component, suggesting for the first time that SNW/SKIP may act by linking transcription initiation and splicing (1). Our laboratory was also the first to analyze, using gene targeting and site directed mutagenesis, the essential function of SNW/SKIP in unicellular eukaryotes. We also implicated SNW/SKIP in sequestering a novel sub-type of cyclophilins (peptidyl-prolyl cis-trans isomerases; 2). According to the current views, SNW1/SKIP binds to promoter regions during transcription initiation, escorts RNApolII during transcription elongation, participates in splicing, and affects polyA addition.

We obtained evidence that the Prp45p, the homolog of SNW1/SKIP in *S. cerevisiae*, affects splicing fidelity – the degree to which the spliceosome adheres to a strict sequence consensus (5). Specifically, we found that *prp45*(1–169) genetically interacts with alleles of NTC members *SYF1, CLF1/SYF3, NTC20*, and *CEF1*, and 2nd step splicing factors *SLU7, PRP17, PRP18*, and *PRP22*. Cwc2p-associated spliceosomal complexes purified from *prp45*(1–169) cells showed decreased stoichiometry of the helicase Prp22p involved in 2nd transesterification, suggesting its deranged interaction with the spliceosome. *In vivo* splicing assays in *prp45*(1–169) cells revealed that branch point mutants accumulated more pre-
mRNA whereas 5' and 3' splice site mutants showed elevated levels of intron-exon 2 intermediate as compared to wild-type cells. Notably, the expression of Prp45(119–379)p in prp45(1–169) cells restored Prp22p partition in the Cwc2p-pulldowns and rescued temperature sensitivity and splicing phenotype of prp45(1–169) strain. Our data suggest that Prp45p contributes, in part through its interaction with the 2nd step-proofreading helicase Prp22p, to splicing efficiency of substrates non-conforming to the consensus.

Yeast CSL project

We reported on the evolutionarily ancestral existence of transcription factors of the CSL family (CBF1 homologs) in *S. pombe* and other yeasts (6). We subsequently found that these factors operate in fission yeast in an antagonistic manner to regulate the coordination of cell and nuclear division, cell adhesion and colony formation (7). We demonstrated that Cbf11p recognizes specifically the canonical CSL response element GTGA/GGAA in vitro. The deletion of *cbf11*+ is associated with growth phenotypes and altered colony morphology. Furthermore, we found that Cbf11p and Cbf12p play opposite roles in cell adhesion, nuclear and cell division and their coordination. Disturbed balance of the two CSL proteins leads to cell separation defects (sep phenotype), cut phenotype, and high-frequency diploidization in heterothallic strains. Our data show that CSL proteins operate in an organism predating the Notch pathway, which is of relevance to the understanding of (Notch-independent) CSL functions in metazoans.

List of relevant publications (2000-2009)

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Research field, aims and directions

The Laboratory pursues studies of membrane related phenomena in yeast using approaches of functional and comparative genomics. The research is aimed at a better understanding of the molecular mechanisms involved in the control of multidrug resistance in industrially and medically important yeast species.

Individual topics pursued in the Laboratory and principal results

a) Mitochondrial membranes and drug resistance

Mitochondria play important roles in energy transformation, intermediary metabolism and biogenesis of the organelle itself. Using specific *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Candida glabrata* mutants deficient in the synthesis of phosphatidylglycerol and cytochromes, respectively, a functional relationship between the structure of inner mitochondrial membrane and the requirement for mitochondrial genome integrity both in the *petite*-negative and *petite*-positive yeast species has been demonstrated (12, 18, 21, 24).

The *PGS1* gene encoding mitochondrial phosphatidylglycerolphosphate synthase has been isolated, molecularly cloned and characterized from both *K. lactis* (11) and *C. glabrata* (21). Unlike *S. cerevisiae* and *C. glabrata*, this gene has been found to be essential in *K. lactis*. The *pgs1* mutations resulted in deficiency of two mitochondrial anionic phospholipids, phosphatidylglycerol and cardiolipin. Mutant cells displayed dysfunctional mitochondria, specific growth defects at higher temperatures and fluconazole resistance pointing to the link among phospholipid homeostasis, mitochondrial functions and drug resistance in yeast (12, 18, 19, 21, 26).

The susceptibility of yeast to strobilurin antifungals including antibiotic mucidin that inhibit mitochondrial electron transport at the level of cytochrome *bc1* complex, has been found to be under the control of both the mitochondrial *cob* gene and the PDR network of nuclear genes involved in multidrug resistance such as *PDR1, PDR3* and *PDR5* (1, 5).
**b) Multidrug resistance in yeast**

Multidrug resistance in yeast results from overexpression of genes encoding drug efflux transporters owing to gain-of-function mutations in transcription factors regulating their expression. After *in vitro* mutagenesis of the *PDR3* gene six single amino acid substitutions were identified in *S. cerevisiae* carboxy-terminal *pdr3* mutants. They increased the activation function of encoded transcriptional activator and led to multidrug resistance due to overproduction of membrane efflux pumps (1, 2). The gain-of-function *pdr3*-9 allele has been successfully used as a dominant selectable marker for the transformation of both laboratory and natural wine strains of yeast (3). Along with gain-of-function, the loss-of-function *pdr3* mutations were also identified and revealed that while Pdr3p can tolerate several substitutions of Asp853 the occurrence of hydrophobic amino acid at this position has an adverse effect on its function (20, 30).

To isolate multidrug resistance reversal agents we developed a yeast based positive screening system (6). After its modification it has been recommended to select the loss-of-function *pdr3* (or *pdr1*) mutants and to identify specific genes whose over-expression or deletion will suppress the expression of multidrug membrane transporters and increase the susceptibility of yeast cells to antifungals (8). The screening of a library of synthetic compounds for modulators of drug resistance using the multidrug resistant *S. cerevisiae pdr3*-9 mutant strain led to isolation of 7-chlorotetrazolo[5,1-c] benzol[1,2,4]triazine (CTBT) which increased the sensitivity to fluconazole of both drug resistant and drug sensitive yeast cells (17). The CTBT mode of action associated with intracellular superoxide production and oxidative stress induction has been elucidated with the combination of chemogenomic and transcriptome analysis (28). Using the yeast based screening system and genome wide transposon mutagenesis two novel genes have been found to be required for drug resistance and cycloheximide-induced *PDR5* transcription. The *RPD3* gene encodes histone deacetylase and the *ROM2* gene is coding for GDP/GTP exchange protein participating in the cell integrity signaling pathway (22).

In attempts to isolate the *K. lactis* counterparts of genes involved in *S. cerevisiae* multidrug resistance several homologues have been isolated, cloned and molecularly characterized. They include *KlrpL28* encoding ribosomal protein responsible for native cycloheximide resistance of *K. lactis* (4), *KlpDr5* encoding drug efflux ABC-transporter (7), *KlpNQ1* encoding drug efflux permease of the MFS superfamily (10, 13) and *KlpDr1* encoding the closest homologue of the *S. cerevisiae PDR1/PDR3* genes (25). The inventory of multidrug transporter genes and transcriptional regulators found in complete genome sequences of seven yeast species has been reviewed. The emphasis has been paid to regulators involved in multidrug resistance (16, 23).

Multidrug resistance is known to be responsible for the failure of the treatment of human fungal infections. *C. albicans* followed by *C. glabrata* and *C. krusei* were found to be the most frequently identified species among vaginal yeast isolates. Almost 13% of them were *in vitro* resistant to fluconazole and some of them were cross-resistant to itraconazole and other azole antifungals (9). Decreased susceptibilities of *C. albicans* vaginal isolates to antifungics resulted from the combination of several molecular mechanisms involving drug efflux and alterations in the structure or cellular amount of Erg1lp (15). On the other hand, only the drug
efflux has been found to be responsible for fluconazole resistance in *C. glabrata* clinical isolates. This opportunistic human pathogen is phylogenetically more related to *S. cerevisiae* than to *C. albicans* (14). Two new gain-of-function mutations, Leu347Phe and His576Tyr, were identified in CgPdr1p transcriptional activator and proved to be responsible for azole resistance and over-expression of the *CgCDR1* and *CgCDR2* genes encoding the main multidrug resistance ABC-transporters in *C. glabrata* (27). Along with the genetic diversity of *C. glabrata* clinical isolates their molecular analysis using multilocus sequence typing and microsatellite marker analysis revealed a common clonal origin of some drug resistant isolates (29).

**List of relevant publications (2000 – 2009)**


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Research field, aims and directions

The yeast’s Saccharomyces cerevisiae lipid metabolism shares many common features with higher eukaryotes and thus S. cerevisiae serves as a suitable model organism to study various aspects of membrane biogenesis. Importantly, recent results implicate defects in lipid biogenesis in the etiology of a number of serious human diseases. Over the past ten years, two research groups of the Laboratory of Membrane Biogenesis have been focused on the control mechanisms governing lipid biosynthesis, transport and turnover.

Individual topics pursued in the Laboratory of Membrane Biogenesis and principal results

Genetic and environmental control of neutral lipid biogenesis in yeast

The levels of neutral lipids are controlled by external and internal factors. We have investigated the role of oxygen in the control of sterol ester synthesis in the yeast S. cerevisiae. We have found that genes encoding two acyl-CoA:sterol acyltransferases (ARE1 and ARE2) form a normoxic/hypoxic gene pair and that their response to oxygen and heme is mediated by differential effect of transcription factors Hap1p and Rox1p. Differential activities and specificities of these enzymes indicate that Are1p activity represents a protective mechanism under conditions of increased influx of unnatural sterols in anaerobic cells (Valachovič et al. 2001; Valachovič et al. 2002). Levels of neutral lipid respond generally to environmental conditions and metabolic fluxes in yeast (Hronská et al. 2004; Beopoulos et al. 2008).

Molecular mechanisms of resistance to antifungal agents

Mechanisms of resistance against two classes of antisterol antimycotics have been studied in collaboration with Karl Franzens University Graz (Austria). A series of mutants conferring resistance to terbinafine (inhibitor of squalene epoxidase) have been isolated and characterized on molecular levels. All isolated mutants have been identified as single amino acid substitution in the coding region of ERG1 gene (Klobučníková et al. 2003; Leber et al. 2003). Mapping of the terbinafine-resistant and terbinafin–hypersensitive mutations in the ERG1 gene enabled to design a model of the 3D structure of yeast squalene epoxidase (Ruckenstuhl et al. 2007).

We have isolated two mutants of S. cerevisiae showing selective resistance to one of two clinically relevant polyene antimycotics - nystatin and amphotericin
B. Nystatin resistance in the first mutant has been linked to the changes in sterol spectrum caused by a single base substitution in the ERG3 gene. The molecular characterization of the amphotericin B-resistant mutant is currently in progress. Selective resistance to one of these two structurally similar antimycotics indicates that amphotericin B and nystatin may differ in the mechanisms of their activity (Hapala et al. 2005).

**Regulation of yeast phospholipid biosynthesis and turnover**

Phospholipid biosynthesis and its turnover are the two processes that play a major role in maintaining the optimal membrane phospholipid composition. In collaboration with S. Henry (Carnegie Mellon University, Pittsburgh, USA) we have investigated the response of transcription of genes encoding enzymes involved in phospholipid biosynthesis to nitrogen and carbon limitation. Regulation of phospholipid biosynthesis on the transcriptional level by Sec14p and its homologues was also studied (Holíč et al. 2004). The results supported the model in which high phosphatidic acid levels lead to derepression of the genes of phospholipid biosynthesis.

Phospholipid turnover in addition produces important lipid derived signalling molecules. In collaboration with J. Vogt (Duquesne University, Pittsburgh, USA) we have described the function of the yeast genome open reading frame YKL110c as a glycerophosphocholine phosphodiesterase (Fisher et al. 2005) and established its function in lipid metabolism. Recently, we have described a novel degradation mechanism in the cardiolipin biosynthetic pathway regulating the amount of phosphatidylglycerol via a phospholipase C type degradation mechanism (Šimočková et al. 2008).

**Phosphatidylinositol transfer protein and its homologues in yeast**

Major yeast phosphatidylinositol transfer protein, Sec14p, is involved in protein secretion and regulation of lipid synthesis and turnover. Our research was focused on determining subcellular localization of yeast Sec14 homologues and their involvement in regulation of phospholipid turnover (Fig.1) (Schnabl et al. 2003). An important observation was that phosphatidylcholine (PC) transfer ability of Sec14p is not required for its essential function(s) in living cells, however, yeast cells having PC transfer deficient Sec14p(D115G) as a sole Sec14p display multiple regulatory abnormalities (Tahotná et al. 2007). In addition, our group published two fundamental review articles on the subject (Griač et al. 2006; Griač 2007).

**List of relevant publications (2000-2009)**

Research field, aims and directions

Our studies on the yeast *Saccharomyces cerevisiae* mainly concerned (i) the analysis of plasma membrane microdomains (see ref. 5, 10, 11, 18, 23, 24, 29, 30), (ii) phenotypic analyses of mutants in chromatin-remodeling factor ISWI (see ref. 2, 9, 22, 27), and (iii) the cellular functions of the translation initiation factor eIF3 (see ref. 1, 12, 16, 19, 33, 35, 39).

Individual topics pursued in the Laboratory and principal results

*Plasma membrane compartmentation*

In cooperation with the Institute of Cell Biology and Plant Physiology at Regensburg University, we obtained important results with a key impact on understanding plasma membrane arrangement in eukaryotic cells. With the use of fluorescent markers attached to various plasma membranes proteins, we showed that the plasma membrane of *S. cerevisiae* is subdivided into at least two stable lateral compartments. One consists of 50 to 80 patches of about 300 nm in diameter is called MCC (Membrane Compartment of Can1, the arginine permease). The other one- MCP (Membrane Compartment of Pma1, the H⁺/ATPase) fills the space in between the MCC patches. Some other PM proteins, like Hxt1 or Gap1 are distributed homogeneously. The distribution of proton symporters in MCC can be affected by, e.g., lipid composition or the plasma membrane energization, the distribution of other proteins is resistant to these effects. Recently we have documented that the dwelling in the MCC protects its residents against endocytosis. The physiological importance of the compartmentalization of the plasma membrane is under intensive study in our group.

*Functional analysis of Isw2 protein*

Strains of *Saccharomyces cerevisiae* lacking Isw2, the catalytic subunit of the Isw2 chromatin remodeling complex, show the mating type independent activation of the cell wall integrity (CWI) signaling pathway. Since the CWI pathway activation usually reflects cell wall defects, we searched for the cell wall-related genes changed in expression. The genes *DSE1*, *CTS1* and *CHS1* were upregulated as a result of the Isw2 absence, according to our previously published gene expression profiles. Western blot analyses of double deletion mutants, however, did not indicate contribution of the chitin metabolism-related genes *CTS1*
and CHSI to the CWI pathway activation. Nevertheless, deletion of the DSE1 gene encoding a daughter cell-specific protein with unknown function suppressed the CWI pathway activation in isw2Δ cells. In addition, deletion of DSE1 also abolished the budding-within-the-birth-scar phenotype of isw2Δ cells. Plasmid-driven overexpression proved that deregulation of the Dse1 synthesis was also responsible for the CWI pathway activation and manifestation of the budding-within-the-birth-scar phenotype in wild-type cells. The overproduced Dse1-GFP localized to both sides of the septum and persisted in unbudded cells. Although the exact cellular role of this daughter cell-specific protein has to be elucidated, our data point to involvement of Dse1 in bud site selection in haploid cells.

**Stress-induced rearrangement of translation machinery**

In higher eukaryotic cells exposed to various environmental stresses translation initiation factors accumulate in cytoplasmic aggregates, called stress granules. The stress granules were identified in heat-stressed fission yeast but until now their formation has not been reported for the budding yeast *Saccharomyces cerevisiae*.

We have constructed various *S. cerevisiae* strains expressing fusion proteins tagged with GFP and/or mRFP from their chromosomal sites and analyzed redistribution of translational factors, ribosomal proteins, mRNA binding proteins and mRNA under various stresses. Together, our data indicate that the protein accumulations induced by robust heat shock represent dynamic structures of some translational components resembling stress granules of higher eukaryotic cells (1).

**List of relevant publications (2000-2009)**


**Fig.**: Bundles of F-actin visualized by Abp140GFP in *Saccharomyces cerevisiae*. Photo J. Hašek
Research field, aims and directions

- Cell cycle and cytoskeleton in yeasts
- Proliferation of yeasts and cytoskeleton
- Role of cytoskeleton in transport
- Microtubules and actin
- Microtubule proteins (tubulin, MAP1, MAP2,
- Molecule transport along microtubules, "road blocks" and their role in ageing

Individual topics pursued in the Laboratory

- Cell cycle mutants of *Yarrowia lipolytica*
- Regulation of cell proliferation
- Studies on transport into and out of the cell nucleus
- Visualization of actin cytoskeleton in yeasts by means of immuno-electron microscopy
- The role of calmodulin in karyokinesis and in cytokinesis
- Relationships between cytoskeleton and calmodulin in yeasts
- The role of calmodulin in cell cycle

List of relevant publications (2000-2009)

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Research field, aims and directions

The research is focused on the cell biology of human pathogenic yeasts and fungi. The aims are (i) to identify cytoskeletal structures in human pathogenic yeasts and fungi and (ii) to study cytoskeleton structures as potential targets for cytoskeleton inhibitors.

Individual topics pursued in the Laboratory and principal results

The cytoskeleton in human pathogenic yeast microorganisms

Our research projects are directed at the description of actin and microtubule cytoskeletons in selected strains of human pathogenic yeasts and fungi and how microtubules and F-actin are involved in budding, fission, hyphal morphogenesis and conidiogenesis. We identified actin structures and microtubules in several human potential pathogens (*Cryptococcus neoformans, Aureobasidium pullulans, Fellomyces fuzhouensis, Cryptococcus laurentii*) belonging to ascomycetous and basidiomycetous yeasts and yeast-like microorganisms, and compared them to the previously described cytoskeletal structures in of *Saccharomyces cerevisiae, Schizosaccharomyces pombe* and *Schiz. japonicus*. In *Malassezia pachydermatis* only the microtubules were detected, but not actin. However, the presence of actin in *Malassezia* was proved by immuno-electron microscopy. From other human fungal pathogens studied the ascomycete *Sporothrix schenckii* showed microtubules, but not actin, zygomycete *Rhizopus nigricans* and *Rh. stolonifer* showed microtubules and actin patches, basidiomycete *Filobasidiella neoformans* had actin patches, cables, rings and microtubule cytoskeletons (unpublished data). Our results proved that the cytoskeleton is involved in budding, bud-fission, hyphal growth and conidiogenesis of human yeast and fungal pathogens (1, 2, 5, 6, 7, 8 and 9).

The cytoskeleton as a potential target for the study of effects of antifungal agents on the morphogenesis of human pathogenic yeasts

We investigated the cytoskeleton of human fungal pathogens as a potential target of inhibition of morphogenesis using inhibitors of microtubules - „laboratory“(Methyl benzimidazol-2-yl-carbamate, Thiabendazole, Nocodazole),
“clinical” (Vincristine, Vinblastine, Paclitaxel, Docetaxel), actin inhibitors (Cytochalasin D, B, A, Latrunculin A) and “spindle” poison (Chloropropham). The objectives were to find out whether microtubule or actin inhibitors will inhibit budding, fission, hyphal growth and conidiogenesis, or whether they will selectively block specific morphologic forms (conidia, yeast, filamentous) including dimorphic fungi. *Aureobasidium pullulans, Fellomyces fuzhouensis, Cryptococcus neoformans, Filobasiidella neoformans, Rhizopus nigricans* and controls *S. cerevisiae, Schiz. japonicas, S. pombe* were used. All laboratory microtubule inhibitors were effective; we found conditions, similar to laboratory inhibitors, for clinical inhibitor Vincristin to inhibit budding, hyphal growth and conidiogenesis (unpublished data). However, with all microtubule inhibitors the inhibition was only transient, as resistant cells appeared in the cultures, similarly as in other clinical inhibitors. Cytochalasins A, B, D were not effective with the exception of *Schizosaccharomyces*; Gabriel et al., Microbiology 144, 2331-1344, 1998). Latrunculin A caused effective inhibition of budding, fission, hyphal growth and conidiogenesis by disrupting actin cytoskeleton; microtubules persisted, but nuclear division and cytokinesis were inhibited. The most efficient inhibition occurred by combining a microtubule and an actin inhibitor: both actin and microtubules disappeared, mitosis, cytokinesis, conidiogenesis, hyphal growth and proliferation of cultures were inhibited and no resistant cells appeared (10; Kopecká et al., *Chemotherapy*, accepted; and unpublished data). Chloropropham effectively inhibited all yeasts and fungi tested (unpublished data). Ultrastructural study of actin mutant of *S. cerevisiae* demonstrated that actin cables are necessary for (i) correct spatial positioning and orientation of secretory pathway to the bud and septum, and for (ii) vectorial movement of vesicles of the secretory pathway along the actin cables to the bud and septum (11).

Our investigation brought new priority findings in basic research on the cytoskeleton as a new target of inhibition of morphogenesis of yeast human pathogens (3, 4, 5 and 10), which may show the way to a new antifungal strategy.

**List of relevant publications (2000-2009)**

7. Gabriel M., Kopecká M., Yamaguchi M., Svoboda A., Takeo K., Yoshida S.,
Ohkusu M., Sugita T., Nakase T.: Cytoskeleton in the unique cell reproduction
by conidiogenesis of the long neck yeast Fellomyces (Sterigmatomyces)
8. David M., Gabriel, M. Kopecká M.: Microtubular and actin cytoskeletons
and ultrastructural characteristics of the potentially pathogenic
basidiomycetous yeast Malassezia pachydermatis. Cell Biol. Int. 31, 16-23
(2007a).
9. David, M., Gabriel M., Kopecká M.: Cytoskeletal structures, ultrastructural
characteristics and the capsule of the basidiomycetous yeast Cryptococcus
10. Kopecká M., Gabriel M.: Microtubules and actin cytoskeleton of potentially
pathogenic basidiomycetous yeast as targets for antifungals. Chemotherapy 55,
278-286 (2009).
11. Yamaguchi M., Kopecká M.: Ultrastructural disorder of the secretory pathway
in temperature-sensitive actin mutants of Saccharomyces cerevisiae. J.

Fig.: Freeze-fracture and transmission electron microscopy of an actin mutant of
Saccharomyces cerevisiae act1-2 DBY 1695. Visible are numerous secretory
vesicles accumulating inside the cell. Photo M. Kopecká and M. Gabriel.
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Research field, aims and directions

Homologous recombination plays a key role in the maintenance of genome integrity, especially during DNA replication and the repair of double-stranded DNA breaks. Homologous recombination reactions entail the assembly of a Rad51 nucleoprotein filament on ssDNA that mediates the formation of joints between sister chromatids or homologous chromosomes. However, Rad51 protein requires the help of recombination mediators that are able to overcome the inhibitory effect of ssDNA binding factor RPA. The main goal of our research is to characterize the role of recombination mediators as well as other factors that regulate homologous recombination. In addition, we also focus on reconstitution of DNA repair synthesis following Rad51-mediated reaction forming D-loop structure as well as resolution of recombination and replication intermediates.

Individual topics pursued in the Laboratory

The research in our laboratory has focused on homologous recombination (HR). Pivotal to this mechanism is a role of Rad51 protein that has the ability of searching for a DNA homologue to generate the D-loop structure. The assembly of the Rad51 presynaptic filament is facilitated by several recombination mediator proteins. In yeast, this role is served by Rad52 protein. We have identified a new DNA binding domain within its carboxyl-terminus. Importantly, we showed that the Rad52 C-terminal portion alone can promote Rad51 presynaptic filament assembly. In addition, we identified the middle portion of Rad52 associating with DNA-bound RPA, enhancing recombination mediator activity and contributed to mapping RPA-Rad52 interaction. In human, we have defined the role of BRCA2 as a recombination mediator nucleating hRad51 onto ssDNA and utilizing replication protein A-coated ssDNA as a recombination substrate. We also have preliminary data that show dissection of individual domains within BRCA2 protein required for mediating Rad51-filament formation as well as possible role of DSS1.

Despite the role of HR contributing to the elimination of DSBs, it must be tightly regulated to prevent untimely events that could interfere with other DNA repair or replication. We have been collaborating on characterization of Srs2 anti-recombinase activity and found that a physical interaction between Rad51 and the C-terminal region of Srs2 triggers ATP hydrolysis within the Rad51 filament,
causing Rad51 to dissociate from DNA. This allosteric mechanism explains the biological specialization of Srs2 as a DNA motor protein that antagonizes HR. We have also contributed to an identification of another mechanism requiring Mph1 helicase, which attenuates HR and crossing over by unwinding Rad51 D-loop structures. Based on cell biology data we have suggested that Srs2 antagonizes Rad52 in the formation of Rad51 filaments. This was further supported by the ability of Rad52 protein to suppress the inhibitory effect of Srs2 on Rad51-mediated strand exchange. Furthermore, the Rad51 mutants, originally isolated as Rad52-interaction deficient mutants, appeared also defective in the interaction with Srs2, suggesting that these proteins compete for the same interaction region. As expected, these Rad51 mutants show resistance to the action of Srs2 as well as inability to overcome the RPA inhibition on Rad51-mediated strand exchange.

Accordingly, a Srs2 mutant protein, that fails to interact with Rad51, is not sufficient for anti-recombinase function in vitro as well as in vivo. Based on this antagonistic role of Rad52 and Srs2, we have proposed a “quality control” mechanism of HR. Recently, we have identified SUMOylation of Rad52 attenuating its DNA binding and strand-annealing activity as a possible regulator of HR.

Resolution of recombination intermediates generated by Rad51 has proven to be a cause of lethal phenotypes in several genetic backgrounds. We showed strong stimulation of Mus81/Mms4 nuclease activity by Rad54 protein that normally helps Rad51 in homology search. This enhancement does not require Rad54 ATP hydrolysis. Rad54 acts by targeting the Mus81/Mms4 complex to its DNA substrates and we demonstrate that this nuclease enhancement is evolutionary conserved.

List of relevant publications (2000-2009)

ATP-dependent protease group
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Research field, aims and directions

Proteases in mitochondria, their function and role in mitochondrial homeostasis represent the major goal of research in the Department of Biochemistry.

Mitochondria are essential components of the cells because they generate most of the cell's source of chemical energy, adenosine triphosphate (ATP). In addition to supplying cellular energy, mitochondria play role in other processes, such as cellular differentiation, control of the cell cycle and cell growth, signaling and cell death. Mitochondrial disorders are connected with a variety of diseases such as myopathy, diabetes, neurological disorders, multiple endocrinopathy, or a variety of other systemic manifestations.

Proteases play crucial role in the maintenance of fully functional mitochondria. Processing peptidases cleave the mitochondrial targeting pre-sequences, enabling the proper folding and distribution of transported mitochondrial proteins. ATP dependent proteases are important for the degradation of miss-assembled and mis-folded and oxidatively damaged proteins within the mitochondria. They play important role also in maintenance of mtDNA integrity, replication and translation.

We characterized several Lon proteases (yeast, maize, rat liver and human) in mitochondria. These studies included investigation of their role in mitochondrial homeostasis, characterization of their inhibitors and structures, finding endogenous substrates and explanation of the way of their recognition and degradation. The system of Lon binding to mtDNA was described in a close cooperation with C. Suzuki, UMDNJ USA. We also participated in mitochondrial processing peptidase studies in Dr. Janata’s laboratory at the Institute of Microbiology, ASCR in Prague.

Individual topics and principal results

Structure and biochemical characteristics of the yeast Lon protease

Lon protease belongs to the family of ATP-dependent proteases and represents a unique protease, where ATPase domain and proteolytic domain are localized on the same subunits. EM studies of the yeast Lon protease in close cooperation with Prof. Schatz’s group and Prof. Stahlberg (Biocenter University of Basel, Switzerland) showed that this protease formed a heptameric ring similar to the later described E.coli Lon protease. Lon protease structure was stabilized by the presence of ATP and a nonhydrolyzable ATP analogue.
Fig.: ELM analysis of Lon proteases. (A1) (A2) Saccharomyces cerevisiae Lon non-symmetrized average images with diameters of the ring and central hole (11 and 2.5 nm, respectively). The leg-like structures are less contrasted in the average image because of their high flexibility. (B) (C) Correlation averages of E. coli Lon wild type and E. coli Lon E614K mutant with the diameters of the ring and central hole of EcLon (11 and 3 nm, respectively) and of EcLon E614K mutant (12 and 4 nm, respectively) (Park, S.C., Jia, B., Yang, J.K., Van, D.L., Shao, Y.G., Han, S.W., Jeon, Y.J., Chung, C.H., Cheong, G.W., 2006. Mol. Cell, 21, 129).

Yeast Lon protease (yLon) similarly to other Lon proteases requires ATP and magnesium for protein degradation. It is a serine protease but typical serine protease inhibitors like PMSF, TLCK, TPCK did not efficiently inhibit yLon activity, while ATPase inhibitors vanadate and EDTA totally blocked its activity. Biochemical characteristics and structure of yLon resembled those of maize, rat liver and human Lon we have also studied.

Role of the ATP dependent Lon protease in yeast mitochondria

The Lon protease plays an essential role in mitochondrial function by maintaining respiration, eliminating non-assembled subunits of mitochondrial complexes and stabilizing the S. cerevisiae mitochondrial genome (1).

Disruption of the S. cerevisiae LON gene resulted in an accumulation of inclusion bodies inside the mitochondria, respiration-deficient mutants, and a nonfunctional, deleted mitochondrial genome (1, 9). The respiratory-deficient phenotype could not be restored by either an ATPase mutant or a protease mutant of Lon. On the other hand, coexpression ATPase- or protease-containing regions of Lon did sustain wild-type growth although when these regions were expressed
separately as truncated proteins no restoration was observed. So it seems that Lon ATPase and protease domains can coexist and cooperate in the cell (9).

**Lon protease and mtDNA**

Yeast *S. cerevisiae* (yLon) was shown to be important for the mitochondrial DNA (mtDNA) integrity. We have found that the activity of yLon was stimulated by the presence of DNA, but we were unable to detect the yLon DNA binding, most probably because of high instability of the yeast protease. On the other hand in close cooperation with the Carolyn Suzuki UMDNJ USA much stable human mitochondrial Lon (hLon) was characterized as DNA binding protein. Affinity to mtDNA was supported by substrate binding but was abolished by the presence of ATP (7). mtDNA binding protein like POLG and Twinkle were found to coimmunoprecipitated with the hLon (7). Purified hLon was shown preferentially bind to a mtDNA sequence overlapping the light strain promoter (LSP) region of human mtDNA predicting potential role of hLon in mtDNA metabolism, damage, and repair (4).

**Endogenous substrates and their recognition and degradation**

Lon protease recognizes and cleaves two types of proteins: unfolded proteins that are produced under stress conditions and folded proteins whose level must be tightly controlled. Many of the mitochondrial proteins function in complexes. Proper assembly of these complexes must be precisely controlled and non-assembled subunits have to be removed or else they form aggregates that are harmful to the organelles. Lon protease is responsible for the degradation of several subunits that are normally assembled in complexes, such as the α, β, γ subunits of the F1 ATPase complex, some ribosomal proteins, and mitochondrial processing peptidase subunits (MPPα, MPPβ) (5). Yeast and rat liver MPPα and MPPβ were degraded by both the yeast and the human Lon in an ATP-dependent manner only when they were not assembled into functional complexes; complex formation completely blocked their degradation (5).

The generally accepted degradation mechanism for ATP-dependent proteases involves the binding of the substrate to the ATPase complex (domain), followed by subsequent unfolding and translocation to the proteolytic cavity where it is processively degraded (2). In agreement with other ATP-dependent Lon substrate studies, our studies demonstrated that the initial Lon mediated cleavage generally occurred between hydrophobic amino acids positioned at internal sites within the primary sequence of the protein, without apparent regard for the secondary structure. However, a detailed analysis of the cleavage sites of MPPα and StAR showed that, although the initial Lon cleavage sites were hydrophobic, they were exposed at the surface of the protein within strongly polar environments. It seems that Lon protease recognizes and initially cleaves the endogenous substrates based on their surface structural features. These results suggest an alternative degradation mechanism for Lon protease (5).
**Mitochondrial processing peptidase**

We have also participated in mitochondrial processing peptidase characterization in Dr. Janata’s laboratory at the Institute of Microbiology, Academy of Sciences of Czech Republic. C-terminus of the α subunit of this crucial mitochondrial peptidase (α-MPP) was found to be an important stabilizing element. The data obtained from tryptophan fluorescence measurements have indicated considerable complicity of the extreme C-terminus of yeast α-MPP in the overall subunit conformational change (6). Analogue of the yeast processing peptidase was found and characterized also in *Trichomonas vaginalis* and *Giardia intestinalis* (3) and we contributed to this study as well.

**List of relevant publications (1998-2009)**


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Research topics

- Isolation, selection and characterization of autochthonous yeast *Saccharomyces cerevisiae*. Application of these strains to winemaking practice to achieve relevant quality and enhance originality and authenticity of wine.
- Influence of yeast strain on sensory profile of wine: production of miscellaneous volatile compounds by yeast strains according to conditions of fermentation.
- Influence of *Saccharomyces cerevisiae* strain on antioxidative activity of wine. Research into the relation between antioxidants in wine and yeasts used for fermentation.

Current research

Our research is focused on isolation of new autochthonous yeast strains potentially useful in practical winemaking. The objective of our project is to collect autochthonous pure cultures of noble wine yeast *Saccharomyces cerevisiae* from different vineyards of Slovak vineyard regions and study their influence on individual sensory properties of wine. Beside characterization of impact of the yeast strain on the chemical essence of color, aroma and flavor of young wine our aim is to study the effect of yeast on the profile of antioxidants, mostly from the group of phenol substances. We also assess the participation of individual phenol compounds in the antioxidant activity of wine.

Our most important objective is to harmonize the functions of pure cultures of *S. cerevisiae* with the goal to produce quality wine that has properties typical for selected locality and region and is beneficial for our health.

Innovation and originality of project lie in the following items and assumptions. Today, commercial preparations of active dry yeast are used too often and resulting wines give the same uniform impression. To preserve originality and authenticity of Slovak wines it is very important not only to use native must, which is typical for each growing region; also the participation of authentic strains of wine yeast, which is part of the natural micro-flora of vine, is very important. These strains can be isolated and applied similarly as commercial dry yeasts. In order to
preserve original local character of wine the following simple principle can be used – the application of yeast strains that originate from the same viticulture region or vineyard as the fermented must. Research into the influence of \textit{S. cerevisiae} on antioxidant properties of wine makes application of this species wider and allows for production of quality wine with high antioxidant activity and preventive healing potential.

**List of relevant publications**


**Fig.:** Fluorescent staining of actin by rhodamin-phalloidin in \textit{Schizosaccharomyces pombe}. Photo M. Gabriel, Masaryk University, Brno, Czech Republic
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Research field, aims and directions

The main topics of Department of Food Chemistry and Biotechnology are focused on PCR-based techniques used for monitoring of microflora during the winemaking process. This topic is studied in co-operation with the Institute of Chemistry, Slovak Academy of Sciences, Bratislava (Dr. E. Slavíková, Dr. R. Vadkertiová). Attention is also focused on potential use of selected waste substrates from food industry and other types of plant waste for the production of selected yeast metabolites. Microbial pectolytic and cellulolytic enzymes are studied in co-operation with the Institute of Chemistry, Slovak Academy of Science, Bratislava (Dr. E. Stratilová).

Red yeast research group started its experimental work in 1998 after re-establishment of Faculty of Chemistry BUT and opening of new biotechnology laboratories. Research is focused on a complex metabolic study of red yeasts exposed to different types of stress. Cell response is studied simultaneously on genomic, proteomic and metabolomic level. To increase the yield of carotenoid pigments, several types of environmental changes as well as nutrition stress were applied. Some topics are studied in co-operation with the Institute of Chemistry, Slovak Academy of Science, Bratislava (CCY, Dr. E. Breierová) and Faculty of Biochemical Technology STU, Bratislava (Dr. M. Certík).

Another part of yeast research is focused on genotoxicity and antimutagenicity tests using unicellular yeast test system *Saccharomyces cerevisiae* D7.

Individual topics pursued at the Department of Food Chemistry and Biotechnology and principal results

*Composition of microflora during winemaking* (J. Omelková, D. Vránová)

Molecular biological methods are employed for taxonomical identification of yeasts involved in wine fermentation. New taxonomic procedures were introduced for easy and fast identification of microflora during winemaking. One of them is the PCR-RFLP method. Conventional identification methods are time-consuming, while molecular methods based on PCR enable fast and precise identification of microorganisms. The study is concentrated on differences between
the population of yeast arriving from wine from ecological production and standard production. In the near future, we would like to aim our activity at the problem of influence of particular yeasts on wine quality (8, 20, 25, 27).

Use of industrial waste substrates to production of fungal metabolites (J. Omelková, I. Márová)

This part of experiments deals with the utilization of plant waste (produced during industrial processing) and humic acids, along with yeasts and yeast-like microorganisms, for the production of enzymes that have further beneficial industrial utilization. Special attention is concentrated on production of pectolytic and cellulolytic enzymes (4-6, 17, 26). Further, complex waste substrates are tested for production of carotenoid-rich biomass (29).

Production of carotenoids by red yeasts – metabolomic analysis (I. Márová)

A set of different red yeasts of the genes *Rhodotorula, Sporobolomyces* and *Cystofilobasidium* were tested for the production of carotenoids in optimum conditions and under some types of exogenous stress: osmotic stress, oxidative stress, UV irradiation and heavy metal stress. Induction of carotenoid production was observed in some stress conditions, mainly in *Rhodotorula glutinis* CCY 20-2-26 strain (7, 12). The role of carotenoid in red yeast stress response was also studied (14, 28).

Production of carotenoid pigments and ergosterol was analyzed by RP-HPLC/PDA/ESI-MS after extraction and saponification (15, 21). High-performance liquid chromatography is regarded as the preferred method for the separation, identification, and quantitation of carotenoids found in biological matrices. Unfortunately, complex mixtures of carotenoids, many of which are closely related structurally, are often present in biological matrices, rendering unequivocal identification by HPLC using retention time and fixed wavelength data alone unacceptable. The advent of photodiode array detection, allowing for continuous collection of spectral data during HPLC analysis, has provided a powerful tool for carotenoid research. However, tentative identification of carotenoids using HPLC with photodiode array detection requires retention time measurement, complete chromatographic resolution of absorbing species so that spectrophotometric data for each analyzed substance alone are observed, and comparison of UV/VIS spectra and retention times with those of authentic standards. Mass spectrometric and tandem mass spectrometric analyses, which provide molecular weight and characteristic fragmentation patterns, may then provide final confirmation of individual carotenoid identities when used in conjunction with retention and spectral characteristics (18, 19).

Regulation of carotenoid-rich biomass production

Carotenogenic yeasts are considered to be ubiquitous due to their worldwide distribution in terrestrial, freshwater and marine habitats, and to their ability to colonize a large variety of substrates. They can assimilate various carbon sources, such as glucose, xylose, cellobiose, sucrose, glycerol, sorbitol, etc. For this reason, various waste materials were used as cheap substrates for their cultivation (29).

Under stress conditions red yeast accumulates a higher quantity of carotenoids. This is of interest for biotechnology. The use of this stressed biomass
in feed industry could have positive effect not only in animal and fish feeds because of a high content of physiologically active substances, but it could influence the nutritional value and organoleptic properties of final products for human nutrition (1). Knowledge of molecular mechanism of stimulation of carotenoid production can then lead to improvement of such biotechnological process (7, 28, 29).

Proteomic and genomic analysis of red yeasts

Proteomic data of red yeasts are very rare. Proteome analysis of Rhodotorula glutinis and Rhodotorula rubra cultivated under optimal as well as stress conditions was performed in this part of the project. Proteins were extracted from yeast cells and analyzed by 2D-electrophoresis. Image analysis was done using BioRad Laboratories 2D software PD-Quest™. Identification of some spots was done using LC-MS/MS and MALDI-TOF/MS/MS. MASCOT 2.0 search engine was used for processing the MS data. PMF was done against NCBI protein database as well as EST Database.

To this time about 25% proteins were identified in R. glutinis proteome using protein databases. These proteins are involved in gene expression, primary metabolism, stress response, etc. Expression of some protein fractions is changed under stress conditions. Significant 2D proteome changes were observed mainly under peroxide stress (underproduction of 247 from 310 evaluated proteins). Relatively well-balanced response was observed in the presence of 2% salt; it seems that R. glutinis cells could exhibit long-term adaptation to this stress factor. With increased salt concentration 67 spots exhibited gradual quantitative changes in the same direction. Proteome changes were relatively well related to metabolome changes and carotenoid production (21-24).

The karyotype of red yeasts has not yet been described. The main problem is accompanied by successful isolation of intact DNA from yeast cells. In this project, protocols for chromosomal DNA isolation from Rhodotorula sp. and Sporobolomyces sp. were optimized. Then, yeast chromosomes were analyzed by pulsed field gel electrophoresis. Our results indicate that many similarities can be found in karyotypes of Rhodotorula glutinis and Rhodotorula rubra. It confirms their genus conjunction. Different profiles were exhibited by Sporobolomyces roseus karyotypes (18, 23).

Use of yeast test system S.cerevisiae D7 in the study of antimutagenity of natural substances (I. Márová)

This part of our research work is focused on study of antimutagenic/genotoxic effects of some natural substances, foods and food components as well as some polymeric materials. We observed possibility to use this indirect test system to analysis of antimutagenic activity based on inhibition of formation of mutant colonies in presence of standard mutagen and natural extract (9, 10).

List of relevant publications (2000-2009)


18. Halienova A., Marova I., Carnecka M., Konecna H., Hanusova V., Hezinoova V.: Proteome and metabolome changes in red yeasts grown under


Microscopic images and streak plates of red yeasts

1. *Rhodotorula glutinis*

2. *Sporobolomyces shibatanus*

3. *Cystofilobasidium capitatum*
Research field, aims and directions

Research of our Yeast Colony Group is focused on the project “Multicellular yeast communities: Signaling, differentiation, and long-term survival” and performed in a close collaboration with the group of Libuse Vachova, Institute of Microbiology, AS CR, Prague, Czech Republic.

In contrast to exponentially growing cultures such as those routinely studied in laboratories, microorganisms in nature exist predominantly within structured multicellular communities capable of differentiation. In these communities, the benefit of individual cells is subordinated to the benefit of populations. Besides complex structures such as biofilms, colonies of bacteria and yeast represent useful tools for studying multicellularity in microorganisms.

We are investigating the molecular mechanisms underlying strategies involved in long-term development, adaptation, and survival of yeast colonies as a model of a multicellular population. These strategies include long-distance signaling via the simple volatile compound ammonia, metabolic reprogramming (involved in colony adaptation), and differentiation (specialization) of yeast cells within colonies. Survival strategies can differ according to environmental conditions; therefore, we are also interested in features that distinguish the laboratory from the natural lifestyle of yeast.

Individual topics pursued in the Yeast Colony Group and principal results

Ammonia signaling and its role in the long-term development of yeast colonies

This direction of our research came from our original finding that unprotonated ammonia acts as a signal molecule and is produced by colonies of various nonrelated yeast species (Palkova et al., Nature 390, 532-536 (1997)). During growth, yeast colonies change the pH of their surroundings from acidic to alkali and vice versa; ammonia production starts at the beginning of the alkali developmental phase. The source of extracellular ammonia appears to be intracellular ammonium (formed from intracellular amino acid stocks, (4)), which is exported by putative Ato ammonium exporters (18). Ammonia induces reprogramming of yeast metabolism to a more economical variant, which enables yeast to exploit intracellular reserves (such as β-oxidation of fatty acids in peroxisomes) and to cope with intracellular stress (5, 7). Ammonia signaling and the related adaptive metabolic changes appear to be important for long-term colony
survival, as indicated by studies performed on colonies of a strain defective in the Sok2p transcription factor (11). sok2 colonies are not able to produce or accept the ammonia signal, and they exhibit defects in long-term survival. Recent studies on putative ammonium exporters Ato (Ato1p, Ato2p, and Ato3p) revealed that ammonia controls expression of ATO genes. All three Ato proteins localize to the plasma membrane and associate with raft-like detergent-resistant membranes (18).

The released ammonia is able to induce colonies of different yeast species to produce their own ammonia, regardless of their current developmental phase. Thus, a colony that starts to produce ammonia induces all colonies in its surroundings to produce their own ammonia. In this manner, all colonies in a territory are able to synchronize their ammonia pulses and consequent development, suggesting that ammonia may function as an alarm molecule notifying of impending nutrient shortage and inducing important metabolic changes that allow adaptation and survival of the yeast colony population (1, 7).

**Cell differentiation and survival of yeast colonies**

There are several indications that cells in distinct areas of multicellular colonies differentiate (3). We have found that cells with features of apoptosis-like programmed cell death (PCD), localize to specific areas of aging *Saccharomyces cerevisiae* colonies (13). Measures of stress (presence of ROS) and PCD markers (exposure of phosphatidylserine, DNA breaks, caspase-like protease, and chromatin fragmentation) revealed that all the markers are detectable in colonies but that they appear at different time-points and as a function of their position within an aging colony. The ammonia signal and related metabolic changes appear to be important for proper colony differentiation and localization of dying cells. The population that finds itself in more propitious areas (i.e., at the colony margin) can exploit released nutrients and thus survives.

**Distinct lifestyle of wild yeast and its domestication under favorable laboratory conditions**

The natural environment rarely provides microorganisms with conditions that enable their growth and reproduction at a maximal rate. Microorganisms in the wild need to be able to cope with, for example, nutrient starvation, temperature changes, and lack of moisture and should be able to survive for extended periods without reproducing (10, 16). We have evidence that colonies and their behavior in nature differ from those under laboratory conditions. *S. cerevisiae* laboratory strains usually form smooth colonies with no strikingly structured pattern, whereas colonies of wild *S. cerevisiae* strains form fluffy structured colonies even in the laboratory (5).

Ultrastructural native electron microscopy and biochemical analyses revealed that cells within fluffy colonies are connected by an abundant glycosylated extracellular matrix that forms a scaffold for the colony. No such material was observed within the smooth colony. The extracellular matrix appears to be important for protecting the colony from harmful environmental factors and from desiccation. Moreover, similar to biofilms, the matrix can form small channels for nutrient and water flow
and chambers and microenvironments for the next generations of cells. The ability of wild yeast to create structured fluffy colonies is switched off when the organisms are transferred to hospitable laboratory conditions. Such “domestication” is accompanied by the loss of the extracellular matrix and extensive changes in gene expression that indicate a complex reprogramming of the yeast lifestyle. (6)

**List of relevant publications (2000-2009)**


Beginnings and aims

Detailed study of ecology of vine yeast and contaminant microflora is needed for ensuring better management of fermentation processes in production facilities in the Slovakia. To achieve this aim, the late Assoc. Prof. Erich Minárik, who founded and led the microbiological laboratory at the Institute of Viniculture since 1950, established an institute collection of yeast (Yeast Collection of the Research Institute for Viticulture and Enology (RIVE GROUP)) that gradually became an establishment with nationwide impact and, at the beginning of 60s, was integrated into the World Federation of Culture Collections. Dr. Minárik used various parts of the vine from different areas of vineyard to isolate single cells, from which he prepared pure cultures. This marked an advent of new philosophy in applied yeast research and winery practice. Nowadays, the Department provides pure cultures of yeasts for the broadest viticulture use.

Research field, aims and directions

The Collection currently contains 350 strains of wine yeasts and yeast-like microorganisms. It is steadily updated by acquiring new biotechnologically significant strains isolated from vine, must and wine from Slovakia. RIVE GROUP is a member of the Federation of Czechoslovak Collections of Microorganisms (FCCM). Our research is focused on a complex study of grape and wine yeasts. Some topics are studied in co-operation with the Institute of Chemistry, Slovak Academy of Sciences, Bratislava (Culture Collection of Yeasts, Dr. E. Breierová), Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Food Research Institute, Bratislava and with the Faculty of Biochemical Technology STU, Bratislava.

Influence of indigenous grape microflora on the sensory properties of varietal wine

We also study non-Saccharomyces yeasts for their production of aroma compounds. These yeasts strains are taken as one of the most important factors contributing to the wine origin. They produce sensorially active metabolites – volatile compounds that are identified by gas chromatography and mass spectrometry.
Yeast isolation from grapes and in different stages of fermentation and their morphological determination

Wine fermentation is a complex ecological and biochemical process involving the sequential action of different yeast species. Apiculate yeasts such as Hanseniaspora uvarum and its anamorphic form Kloeckera apiculata, and oxidative yeasts such as Candida, Pichia, Rhodotorula and Kluyveromyces are the predominant species on the grapes. We isolated and identified various yeast species from sound and damaged grapes, from rachis and must, and compared various grape varieties of Slovak wine regions.

List of relevant publications


Fig.: Yeast isolated from wine must. Photo E. Breierová
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Research field, aims and directions

Both routine yeast detection and species identification in clinical samples and research in medically important yeasts are performed in our laboratory. Close cooperation of these two areas offers several advantages both for the research and routine practice. Our aim is to work for rapid translation of new scientific knowledge into clinical microbiology practice for the benefit of patients. We also strive to supplement this translational research by an excellent basic research focused on medically relevant topics in yeasts.

Individual topics pursued in the Yeast Laboratory and principal results

Hypoxia-response in Cryptococcus neoformans

Ability to adapt to oxygen limitation was identified as a signal for unbudded G2-arrest in this obligate aerobic pathogenic yeast. Furthermore, a role of hypoxia response in cryptococcal virulence has emerged recently. Therefore, we focus on characterisation of cryptococcal hypoxia response aimed at development of a cultivating system that should serve as a model for cryptococcal growth in human tissue. We have also recently identified novel components of the molecular machinery of cryptococcal hypoxia signalling.

Identification and typing of medically relevant yeasts

Recently, the technology of high resolution melting analysis of double-stranded DNA (typically PCR amplicons) is gradually more affordable to routine clinical microbiology laboratories. Therefore, we focus on developing competitive amplification systems which should best cover the whole spectrum of medically important yeast, enabling for their rapid species identification and possibly also strain typing in a convenient automated or semi-automated way. The McRAPD and PCR-HRMA approaches are at the center of our efforts, including development of multi-purpose software QAS useful for quantitative comparison of complex melting data (melting curves).
List of relevant publications (2000-2009)


Multiple budding pattern of *C. neoformans* under severe hypoxia – phase contrast, India ink preparation, TEM

McRAPD (Melting curve of Random Amplified Polymorphic DNA) results in strains of *C. tropicalis* (dark green lines and lanes 2-6 in part C), *C. krusei* (light green lines and lanes 7-11 in part C), *C. pelliculosa* (violet lines and lanes 2-6 in part D), and *C. guilliermondii* (blue lines and lanes 7-11 in part D). Part (A) normalized melting curves, part (B) derivative curves, parts (C) and (D) conventional fingerprints after agarose gel electrophoresis.

Yeast Population Group

Research field, aims and directions

Research of this group is focused mainly on physiology and molecular biology of multicellular yeast populations (mainly colonies) with special emphasis on the roles of stress response systems, membrane transport proteins, metabolic alterations, signalling and programmed cell death in cell differentiation of the populations and their development and aging. We are also comparing strategies of survival used by populations of natural and laboratory yeast strains. One of the main aims is to uncover the processes specific to yeast multicellular lifestyle. The research is performed in close collaboration with the Yeast Colony Group of Zdena Palková, Faculty of Science, Charles’ University in Prague, Czech Republic.

Individual topics and principal results

Ammonia signalling and its role in the long-term development of yeast colonies

Colonies of *S. cerevisiae* pass through three main consecutive developmental phases, which are defined by changes in pH of the medium – two acidic phases separated by alkali, ammonia producing phase. Ammonia serves as a signalling molecule which induces adaptive metabolic changes in colonial cells (1). These changes are important for long-term colony survival, as indicated by studies on colonies of a strain defective in the Sok2p transcription factor. The colonies of *sok2* mutant strain are neither able to produce nor to accept the ammonia signal. Genome-wide expression studies revealed that *sok2* colonies cannot effectively switch on the genes of adaptive metabolism. Moreover, they exhibit unbalanced activity of various stress-defence enzymes (catalases, superoxide dismutases), and the ROS concentration in cells remains high. Due to these attributes, *sok2* colonies were determined to die prematurely. (2)

Cell differentiation during yeast colony development

We have found that cells of multicellular colonies can differentiate depending on their position in the colony structure. Cells possessing some features of metazoan apoptosis (presence of ROS, exposure of phosphatidylserine, DNA breaks, caspase-like protease activation and chromatin fragmentation) are localized to the colony centre. The ammonia signal and related metabolic changes appear to be important for proper colony differentiation and localization of dying cells. Thus central cells being unable to accomplish change of metabolism and remaining stressed are pushed to programmed cell death (PCD). Colony margin cells changing their metabolism to more economical one are healthy and form new generations on
behalf of nutrients released by dying central cells (4, 8). Of the processes predicted to have a role in yeast PCD, we focused on the involvement of caspases - metacastase Mca1p and other caspase-like activities (6).

**Survival strategies of natural and domesticated yeast populations**

Comparison of physiology of structured colonies formed by natural wild *Saccharomyces cerevisiae* strain with that of smooth colonies formed by isogenic domesticated strain, allowed us to propose model of different survival strategies used by wild and laboratory strains in their specific ecosystems. Wild colony is composed of microcolonies embedded in extracellular matrix, which forms channels supplying cells with nutrients and removing waste products and also protecting whole population against environmental attack. It also enables the colony to quickly occupy a territory with a relatively small number of cells and only later to ‘fill’ the ‘chambers’ with new cell generations. Domesticated strains existing in stable laboratory conditions stop to produce protective extracellular matrix saving thus energy. They become tightly attached one to each other within the smooth colony (5).

**Involvement of putative ammonium exporters Ato1p, Ato2p and Ato3p in colony development**

Expression of *ATO* genes is controlled by ammonia. All three Ato proteins localise to the detergent-resistant compartments of plasma membranes. Ato3p–GFP forms stable patches in the plasma membrane; stability of Ato1p–GFP patches is pH dependent; their spreading at low pH is independent on endocytosis. The data suggest that besides the ammonia induction of Ato protein synthesis, pH may rapidly regulate Ato1p function (7). Only cells of several upper layers of microcolony produce Ato1p as was shown by two-photon confocal microscopy approach using the strain producing Ato1p-GFP fusion protein (11).

**Physiological role of MDR transporters during development of yeast population**

Besides their role in removing extracellularly added toxic compounds, transporters Pdr5p and Snq2p (controlled by transcription factor Pdr1p) significantly influence developmental phases and physiology of yeast populations growing in a liquid culture. These proteins appear to be involved in population quorum sensing, which consequently influences Pdr1p level via feedback regulation (9).

Our current studies are supported by grants from Grant Agencies of the Czech Republic, Academy of Sciences, and Ministry of Education.

**List of relevant publications (2000-2009)**


Cell Stress and Ageing Group

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Research field, aims and directions

The keynote research concerns the effects of various types of exogenously applied stress (osmotic, oxidative, transient-metal- and xenobiotic-induced) on cell energetics, membrane functions and structure in yeasts, the function and efficacy of PDR pumps expelling xenobiotics from the yeast cell, and ageing and apoptosis of the cells. A practical spin-off is the study of industrial, in particular brewery, stresses on yeast.

Response of different yeasts to oxidative stress

Schizosaccharomyces pombe, S. cerevisiae and Rhodotorula glutinis differ in their resistance to oxidants (3). Yeast membrane lipids, which are generally poor in PUFA, were found to be subject to perceptible oxidant-induced and antioxidant-suppressed lipid peroxidation (7,11,29,34,35). Doping the S. cerevisiae membrane with poly-unsaturated linoleic and linolenic acids lowers cell survival and increases sensitivity to oxidants. C. albicans with “natural” linolenic acid is considerably less sensitive to the oxidants than S. cerevisiae artificially supplied with linolenic acid. Exposure to anaerobic/aerobic transition or addition of glucose, methanol or cyanide causes strong changes in the redox state of the cells (19). Yeast mutants lacking Cu,Zn-superoxide dismutase and Mn-superoxide dismutase display oxygen-induced growth defects, which are alleviated by antioxidants such as vitamin A, E and quercetin (18,32).

Effect of oxidative stress on S. cerevisiae plasma membrane H⁺-ATPase

Fenton reagents inactivate membrane H⁺-ATPase by metal-catalyzed oxidation, not via lipid peroxidation. The main targets are strategic Cys residues - Cys532, Cys409 and Cys221 (8,20). Inactivated enzyme has a lower ability to generate protonotive force, as measured in vitro in proteoliposomes containing plasma membrane fragments by using the fluorescent probes oxonols V and VI or pyranine, which is suitable for simultaneous monitoring of both membrane potential and ΔpH (4,16,33). The H⁺-ATPase is also inactivated by lysosomotropic agents, which disrupt cell vacuoles (17,25). Commercial biocides such as benzalkonium chloride and 2-phenoxyethanol depolarize and permeabilize the yeast plasma membrane (24). Depolarization alone is not sufficient for cell killing.

PDR pumps of the S. cerevisiae plasma membrane and their regulation

We developed a method for real-time monitoring of the action of S. cerevisiae PDR pumps based on monitoring the uptake of the fluorescent potentiometric dye diS-C3(3), which is expelled from the cells by major PDR pumps Pdr5p and Snq2p (6,10,23,41). The assay showed that the activity of Pdr5p
and Snq2p depends strongly on the growth phase, dropping sharply in diauxic and post-diauxic cells, and also on the type of the carbon source. Stationary-phase respiring cells that have zero pump activity renew the activity on addition of glucose when suspended in a fresh medium while in the original spent medium pump activation/resynthesis does not occur due to depletion of essential nutrients (26,27).

**Replicative ageing and apoptosis in S. cerevisiae**

We elaborated a method of separation of old and young cells from a batch culture of *Saccharomyces cerevisiae*, which is currently used as a model organism of aging and apoptosis (13), and investigated RAS2val19 mutant cells of *S. cerevisiae* as a model of premature aging (12). Centrifugal elutriation was used to separate different size/age categories of yeast cells. Young cells have shown the same lifespan (LS) as the cells from the original batch culture, while the fraction of old cells was senescent (21), with residual LS and with a mean age of 3 - 4 generations. Old cells evinced all markers of apoptosis (5), while young cells exhibited none. Formation of ROS (reactive oxygen species; 12,31) colocalized with mitochondria (2,22). Separated fractions of replicatively old and young cells showed changes in the overall appearance (cell shape and features of cell wall). Microscopic techniques revealed that enlargement of mother cells and growing number of bud scars are accompanied by significant remodeling of cell surface, and can thus be used as additional markers of aging.

**Yeast and brewery technological stresses**

The effects of stress caused by new brewing technologies on the vitality of pitching yeast in beer production were studied by using optimized acidification power (AP) test suitable for assessing the quality of brewery yeast and predicting its performance in wort fermentations. Pitching rate, wort composition, ambient conditions in the cylindro-conical fermentors, and other technological factors were found to vary much more than the vitality of the pitching yeast. A method was developed for a contact-free optical pH measurement of AP, which allows the simultaneous testing of several samples and minimizes the hands-on time in sample processing (28,30,36,38-40,42). Net effect of high wort osmolarity on yeast and the characteristics of beer was determined (46).

**Constituents of cell membrane lipids and other biologically relevant compounds**

In a broad-base study, we identified and characterized a number of odd-numbered very long chain unsaturated fatty acids in yeast that play important roles in the cell wall and plasma membrane (43), e.g. in controlling membrane fluidity (15).

*Two international events were organized; the 5th IMYA (International Meeting on Yeast Apoptosis, September 3-7, 2006, Kutná Hora) and Advanced Course on Cell Biology – Apoptotic processes in yeast and mammalian cells – common and unique features (October 17-19, 2007, Hradec Králové).*
List of relevant publications (2000-2009)


43. Hendrych T., Kodedová M., Sigler K., Gášková D.: Characterization of the kinetics and mechanisms of inhibition of drugs interacting with the *S.


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Research topics
• Diversity of yeasts associated with soil and plant material
• Services: the main activities of the collection are to collect, safe-keep, and provide cultures of yeasts, and to identify and characterize strains for customers

Current project and research
VEGA 2/7031/27 „Diversity of yeasts inhabiting the leaf surfaces with emphasis on their characteristics, activity, and survival“ (2007-2009)

Our research was aimed at exploring the diversity and taxonomy of yeasts and yeast-like organisms isolated from the agricultural soil, their morphological and physiological properties, their ability to survive under stress conditions caused by heavy metals and to produce protective polymeric compounds. Its purpose was to study community of yeast organisms in agricultural soil, to compare it with the diversity of species isolated from forest and grassland soils, and to determine the influence of the soil environment of kind on the incidence of yeasts and their quantity. The representation of the dominant yeast species was found to be very similar in various types of agricultural soil, but the total number of yeasts is tenfold lower than in soils under no till (forest and grassgrown soils). Pesticides also had a significant influence on the reduction of yeast number in soil. Some strains were found to cause structural changes of the lignin macromolecule. Some yeasts were able to accumulate different amounts of heavy metals in dependence on the ability of cells to produce glycoproteins, cell lipids and sterols of different composition and structure, which prevent the uptake of metal ions into the intracellular milieu and cell damage.

Another source of the microorganisms was the leaves of various woody plants and various fruit-trees, their morphological and physiological properties, the influence of environment on their incidence and survival, and the study of some specific attributes that might enable different yeast species to successfully colonize the plant materials in the presence of other microorganisms. The representation of dominant yeast species isolated from the surface of the leaves of various fruit trees was similar, but the frequency of their incidence in the autumn was higher than in the springtime. The surface of woody trees was found to exhibit a greater diversity of yeast species than the leaves of fruit trees. Some yeast strains were able to modify the lignin compounds of the trees and remove some extractive constituents. Also their killer activity was strong. Comparison of yeast populations isolated from
various environments showed that the yeasts isolated from the surface of leaves were the most tolerant.

Some yeasts were able to accumulate different amounts of heavy metals in dependence on the ability of the cells to produce glycoproteins, cell lipids and sterols, which prevent the uptake of the metals and ensuing cell damage. The ability of yeasts to adapt to stress conditions and survive is different and it depends on the production of some biologically active substances in the yeast cell. The influence of heavy metals with biogenic effects and UV radiation on the production of various metabolites of selected yeast species was also studied.

List of relevant publications (2000-2009)


List of publications related to other yeast research


Our long-term goal is to understand the nucleo-mitochondrial relationships that maintain the harmony in eukaryotic cell.

Individual topics pursued in the Laboratory and principal results

Rpm2p

RNase P is an enzyme cleaving 5’ leader sequences from tRNA precursors. RNA part of RNase P from yeast mitochondria is coded by mitochondria and the gene for protein part by nucleus. Protein subunit (Rpm2p) is imported to the mitochondria. Rpm2p is a multifunctional protein which, beside being a part RNase P, is required for the processing of its RNA subunit. Different protein domains determine individual functions: while C-terminal part is involved in the RNase P activity and processing of RNA subunit, N-terminal part is “important for life” (3). In addition, deletion of 100 amino acid residues at the N terminus gives rise to cells unable to tolerate the elimination of mitochondrial DNA. Apparently, Rpm2p is one of the crucial nucleo-mitochondrial communication checkpoints.

Petiteness

Petite-positivity, the ability of organisms to tolerate the elimination of mitochondrial DNA is probably the most important nucleo-mitochondrial communication link. Petiteness is also a thought-provoking paradox, because it emerges and disappears during the evolution. Petite-negative as well as petite-positive species occur among yeasts and protozoa. However, beside the most popular yeast models such as *S. cerevisiae, Kluyveromyces lactis* (7) and *Schizosaccharomyces pombe*, reliable records are outdated. Therefore, the petiteness was examined after the treatment with ethidium bromide in over hundred isolates from the *Saccharomyces/Kluyveromyces* complex. In addition to unequivocal petite-positive and petite-negative phenotypes, a few species displayed a mixed ‘moot’ phenotype, where a major part of the population did not tolerate the loss of mtDNA but several cells did. The genera from postwhole-genome duplication lineages (*Saccharomyces, Kazachstania, Naumovia, Nakaseomyces*) were invariably petite-positive. However, petite-positive traits could also be
observed among the prewhole-genome duplication species (8, 9). Apparently, the tolerance to the elimination of mtDNA requires an adequate capacity of anaerobic metabolism (glycolysis) to supply sufficient amount of energy for the cell proliferation. However, not all facultative anaerobes possess the ability to convert to petites (8, 9).

**Yeast xenomitochondrial cybrids**

Nucleo-mitochondrial interactions, particularly those determining the divergence of species, can be studied by means of xenomitochondrial cybrids (cells where the original mitochondria are replaced by foreign ones from related species). Mitochondria together with their genomes can be transferred to the cells lacking mtDNA as isolated organelles, by protoplast fusion or by mating, if one of the partners carries kar1-l mutation.

Cybrids with *S. cerevisiae* nucleus and mitochondria from five *Saccharomyces* species were prepared. The re-established respiration can be obtained only between related species after a certain period of adaptation and frequently with limited ability to utilize nonfermentable substrates. The main reason of incompatibility appears to be the splicing of the unusual introns and the assembly of cytochrome oxidase complex (2, 6). However, this phenomenon rather reflects the early beginnings of phylogenetic divergence, because with cybrids with *S. paradoxus* nuclei and mitochondria from *S. cerevisiae*, the alien mitochondria are capable of fully substituting original ones (6). The respiratory capacity in less-related species (*S. cerevisiae* - *S. bayanus* “cybrids”) can be recovered only in the presence of chromosomes from both species (6).

**Biotechnology**

To improve the chance of the production of non-alcohol beer with acceptable organoleptic traits, we prepared a mutant strain suitable for the production of beer with reduced ethanol content (10). In the case of mutants in tricarboxylic acid cycle, low ethanol content was compensated by a considerable increase in organic acids (citrate, succinate, fumarate, malate and lactate).

**Taxonomy, Biodiversity and Phylogeny**

During the construction of xenomitochondrial cybrids we observed widespread disparity in taxonomic classification, apparently due to the fact that yeasts used to be characterized by morphological and physiological traits (1,4). Currently, the introduction of molecular methods provides new approaches to yeasts differentiation. Among these, gene sequencing is the most definitive method, with ribosomal RNA gene sequences providing the preponderance of available data (1,4). Therefore, forty isolates belonging to the *Saccharomyces/Kluyveromyces* complex were analyzed for one nuclear and two mitochondrial sequences, and for their karyotypes. The deduced phylogenetic relationships based on the nuclear and mitochondrial sequences were usually similar. The highest degree of polymorphism was observed at the chromosome level (5).

We also investigated the occurrence and diversity of yeasts and filamentous fungi in bryndza, an artisanal cheese prepared from a mixture of sheep and cow milk. Samples collected during four months of the summer production period from two locations (northern and southern parts of central Slovakia)
contained $10^5$–$10^7$ (cfu) yeasts and about $10^2$ (cfu) of molds per gram of wet weight. A novel Geotrichum sp. together with Kluyveromyces (K. lactis/K. marxianus) was identified as the most abundant yeast species by sequence comparison of D1/D2 region from 26S rRNA gene. Other yeasts, such as Candida inconspicua, Candida silvae, Pichia fermentans and Trichosporon domesticum were occasionally found. Further analysis revealed that Geotrichum sp. represents a novel species for which the name Geotrichum bryndzae sp. nov. was proposed (type culture CCY 16-2-1T 5NRRL Y-48450T 5CBS 11176T) (11,12).

**List of relevant publications (2000-2009)**

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Research field, aims and directions
Role of transporters in cell physiology

Individual topics pursued in the Laboratory and principal results

There are five principal topics of research, all of them dealing with the characterization of membrane transport systems and the elucidation of their role in cell physiology.
1) Biochemical, molecular and genetic characterization of membrane transport systems
2) Yeast as a model organism to study transport processes in animal and plant cells; heterologous expression of transporters in Saccharomyces cerevisiae
3) Non-conventional osmotolerant yeasts – physiological and molecular study of their high osmotolerance, development of tools for genetic engineering
4) Role of transporters in Candida virulence and pathogenicity
5) Role of ubiquitin in down-regulation of model proteins in the yeast Saccharomyces cerevisiae

Transport properties and substrate specificities of various systems from yeast, plants and animals involved in transport of alkali metal cation across plasma and organelle membranes have been characterized within the five principal topics. More than ten genes involved in yeast potassium and sodium transport and homeostasis have been cloned and the physiological role of their products characterized in detail.

List of relevant publications (2000-2009)


**Fig.**: Expression of Nha1-GFPp in *S. cerevisiae*. Photo H. Sychrová
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**Research field, aims and directions**  
Physiology and biochemistry of free and immobilised yeast under stress conditions, improvement of beer and ethanol fermentation, low-alcoholic beer production using genetically modified yeast strains.

**Individual topics pursued in the Laboratory and principal results**

*Physiology of free and immobilised yeast under stress conditions*

Very high gravity (VHG) fermentation technology has become popular in distilleries, bio-fuel alcohol production and brewing industry due to reduced energy and capital cost. However, during fermentation of concentrated substrates, the yeast is exposed to extreme conditions of osmotic pressure, toxicity of produced ethanol and shows differences in metabolism accompanied by many negative consequences. An increase in wort gravity results in higher metabolic activity and increased ethanol production, but at certain concentration of dissolved solids in medium, an effect of osmotic pressure, toxicity of produced ethanol, nutrient limitation and enhanced carbon dioxide concentration causes a decrease in yeast viability and the rate and extent of fermentation. The rate of ethanol production increased with increasing wort gravity up to the initial wort concentration of 24 %, reaching the maximum ethanol concentration of 6.2 %, but its attenuation reached only 49 %. The intracellular trehalose accumulation was proportional to the initial wort gravity, at 24 or 30 % wort fermentation it increased 3 or 4.5 times, respectively, compared to 12 % wort fermentation. Trehalose accumulation began after exhaustion of glucose and ceased after the uptake of approximately 65 % reducing saccharides despite increasing ethanol or remaining saccharide concentration in the environment (1).

Immobilization may lower the stress and increase fermentation productivity; but the yeast shows certain modification in physiology, biochemical composition and metabolic activity, and consequently beer flavour. Therefore, we compared osmotic and ethanol tolerance of free and immobilized bottom-fermenting yeast cells and their ability to ferment concentrated wort (up to a concentration of 30 % w/w), and the influence of immobilization on fermentation parameters and beer quality. A significant increase of fermentation rate of VHG wort for yeasts entrapped in gels in comparison to free cells was observed. Using calcium alginate-entrapped yeast, 24 % (w/w) wort was successfully fermented within 8 days. This is half the time needed for fermentation by free yeast. The highest ethanol concentration obtained was 10.5 % (v/v). When the original wort gravity was increased, the specific rate of
ethanol production remained constant (0.16 g.gh⁻¹) and the viability did not fall below 95 % of living cells. The specific rate of ethanol production of yeast immobilized in calcium pectate in 24 % (w/w) wort was at the level of the specific rate of ethanol production of free yeast and yeast adsorbed on cellulose in wort of 16 % (w/w) concentration. Protection of cells against osmotic stress by gel matrix was also confirmed by trehalose measurement. An increase in wort gravity resulted in significantly higher trehalose content in free cells, whereas in entrapped cells trehalose concentration did not increase with increasing initial wort gravity up to the concentration of 24 % (w/w). The maximum intracellular trehalose content in calcium alginate-entrapped yeast was 3 times lower compared to free yeast at 30 % (w/w) wort fermentation. Higher degree of saturation of total fatty acids in the entrapped fermenting yeast compared to the free cells under VHG fermentation conditions correlated positively with ethanol tolerance and with improved fermentation rates. Beer produced from VHG wort by entrapped yeast had suitable diacetyl concentration and higher alcohols-to-esters ratio, in contrast to beer produced by free control yeast (2,3).

**Beer fermentation using immobilized yeast cells**
The primary fermentation was carried out in an up-flow gas lift reactor, the maturation in column reactors with packed-beds. Prior to the maturation a heat treatment of young beer was applied. Yeast cells entrapped in a matrix based on polyvinyl alcohol were employed for both wort fermentation and maturation. The system was stable for 2 months by the residence time of 24 to 36 hours. The produced beer was characterized by excellent quality with a composition and flavour profile similar to that of beer produced by classical batch fermentation (4).

Modelling of ethanol production by yeast, by both free and immobilised yeast cells, is one of the most studied processes. A nonstructured mathematical model was developed to simulate the performance of continuous primary fermentation of lager beer. Substrate consumption was characterised by specific substrate consumption rate and saturation constant. The values of these two parameters were optimized for all four substrates. Inhibition effects of substrates and product were analysed using various inhibition patterns. A good-fitting relationship for maltose inhibition was found, and inhibition constants were calculated (5).

**Improvement of beer production on a brewery scale**
One of the possibilities to make beer production more effective is lowering the time of vicinal diketones (diacetyl and 2,3-pentanedione) reduction. We compared reduction of vicinal diketones in young beers fermented under carbon dioxide pressure of 100 kPa and 50 kPa during the whole main fermentation and in beers fermented without carbon dioxide pressure until extract concentration decreased to 7.5 % (w/w). Lower carbon dioxide pressure accelerated reduction of vicinal diketones and speeded up the end of fermentation (6).

**Low-alcoholic beer production using genetically modified yeast strains**
Production of non-alcoholic beer using mutant strains with a defect in the synthesis of tricarboxylic acid-cycle enzymes were used in both free and pectate-immobilised form, using both batch and packed-bed continuous systems. The beer prepared by mutant yeast cells was characterized by lower levels of total alcohols, with ethanol concentrations between 0.07 and 0.31 % (w/w). The organic acids produced, especially lactic acid, in concentrations up to 1.38 g/l had a strong protective effect
on the microbial stability of the final product and thus the usual addition of lactic acid could be omitted (7,8). A collection of Saccharomyces cerevisiae strains deficient in 14 genes directly encoding enzymes of the TCA cycle and alcohol dehydrogenase (ADH1) has been characterized for the production of beer with a reduced ethanol content. Non-alcoholic beer (with ethanol content below 0.5 % (v/v)) was produced only by the yeast with disruptions in the FUM1, KGD1 and KGD2 genes, corresponding to fumarase and α-ketoglutarate dehydrogenase (9).

List of relevant publications (2000-2009)

Laboratory of Genomics and Biology of Eukaryotic Organelles

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Research field, aims and directions

The research in our laboratory is aimed at the investigation of complex biological phenomena using *Saccharomyces cerevisiae* as well as non-conventional yeast species as model organisms. Specifically, we employ approaches of functional and comparative analysis of yeast genomes to study (i) the structure, function and evolution of telomeres; (ii) replication and maintenance of mitochondrial genomes; (iii) organelle biogenesis and degradation; (iv) nucleo-mitochondrial interactions; (v) mechanisms implicated in the control of mitochondrial integrity; (vi) transport across mitochondrial membranes; (vii) molecular phenomena associated with cellular morphogenesis, and (viii) principles of cognitive biology.

Individual topics pursued in the Laboratory and principal results

*Comparative analysis of yeast nuclear telomeres*
Baker’s yeast *S. cerevisiae* provides an organism of choice for approaching complex biological problems including nuclear telomere structure and DNA replication. In contrast to the high degree of conservation of telomeres observed in most of animal and plant species, yeasts exhibit a high interspecies variability in telomere organization. The sequence variability may result in structural and functional differences. Whereas telomeric chromatin of *S. cerevisiae* adopts a fold-back structure, telomeres of other yeast species may form alternative higher-order architectures. From this aspect, different yeast species may represent naturally evolved alternatives of an accurate telomere maintenance system. For example, research on telomere maintenance in non-conventional yeast species *Kluyveromyces lactis* and *Schizosaccharomyces pombe* opened unique window into understanding the telomere sequence and dynamics and telomere maintenance pathways. On the other hand, telomere studies almost completely ignored other yeast species such as *Yarrowia lipolytica*, *Candida albicans*, *C. parapsilosis*, *C. glabrata*, and *K. waltii*. Nowadays, data from partial or complete genome sequences of several non-conventional yeast species represent unprecedented source of information that may be easily complemented by experimental testing of hypotheses taking advantage of availability of tools for molecular genetics of yeasts. Investigations of structure and replication of telomeres in these species may uncover novel aspects of chromosome dynamics that may be complemented by comparative studies of telomere maintenance pathways at the whole-genome scale.

With this background and in collaboration with Dr. Griffith’s laboratory (University of North Carolina, Chapel Hill, USA) we initiated an investigation of nuclear telomeres in several non-*Saccharomyces* species. In *S. pombe*, the major telomere-binding protein is Taz1 a homologue of human telomeric proteins TRF1 and TRF2. As mammalian TRF1 and TRF2 proteins have been shown to directly bind telomeric DNA to form protein arrays and looped structures, termed telomeric loops (t-loops), the ability of Taz1p to act on fission yeast telomeric DNA in similar ways was examined using purified protein and model DNA templates. Using electron-microscopic analysis we observed not only arrays of multiple Taz1p molecules bound to the telomeric regions, resembling the pattern of TRF1 binding, but also a high proportion of t-loops (63).

Recently we started a comparative analysis of the telomerase complex in various yeast species. There is a high degree of interspecific variation in sequence composition and size (from ~150 nt to >1,500 nt) of the RNA component of telomerase (telomerase RNA; TER). This dramatic divergence has hampered the identification of TER genes and a large-scale comparative analysis of TER sequences and structures among distantly related species. We identified TER genes in several yeast species with relatively large (>20 bp) and nonvariant telomeric repeats, mostly from the genus *Candida*. Within these TERs, we predicted several conserved sequences presumably involved in regulation of telomerase (8). The comparative and mutational analyses of conserved TER elements followed by functional studies currently taking place in our laboratory will provide novel insights into the structure and function of the telomerase ribonucleoprotein complex.

In spite of the dramatic progress in the field of telomere structure, maintenance and evolution there are many unanswered questions, where a combination of several model systems with parallel genetic and biochemical studies can generate extremely valuable insights. For example, we have identified a protein in *Y. lipolytica*, which exhibits biochemical features of a putative ancestral type of
telomeric protein for all eukaryotes (Kramara, J., Willcox, S., Gunišová, S., Kinský, S., Nosek, J., Griffith, J.D., Tomáška, L., unpublished results). Therefore we continue with our effort combining several biochemical, genetic and bioinformatic approaches.

On the origin of telomeres and telomere-maintenance mechanisms: Lessons from yeast mitochondria

The telomeric sequences are restored in most organisms by telomerase. However, in addition to telomerase, there are alternative (ALT) mechanisms for telomere maintenance that may possibly reflect evolutionary earlier and/or independent mechanisms (40 and references therein).

While studying the replication of linear mitochondrial DNA (mtDNA) of *C. parapsilosis* by two-dimensional gel electrophoresis distinct DNA fragments composed solely of mitochondrial telomeric sequences had been detected and their properties were suggestive of a circular conformation. Electron-microscopic analysis revealed the presence of highly supertwisted circular molecules whose sizes corresponded to mono- and multimers of the telomeric tandem repeat unit (n x 738 bp). Similar results were obtained with two other yeast species (*P. philodendri* and *C. salmanticensis*), which possess analogous telomeric structure (58). Subsequently, we have demonstrated that these telomeric circles (t-circles) replicate autonomously via a rolling-circle intermediate (39).

Importantly, since our discovery of t-circles in yeast mitochondria extrachromosomal telomeric DNA has been identified in various systems including ALT cells associated with numerous cancers (62,64, and references therein). Moreover, the occurrence of t-circles in a wide variety of cells from different phylogenetic groups indicates their possible participation in the origin of telomeres (40,64). The discussion about the replication cycle involving t-circles also resulted in a hypothesis explaining a universal phenomenon related to chromosomal ends: intra- and intercellular heterogeneity of telomeres at the level of their structural and functional states. It suggested that this heterogeneity is not a simple by-product of molecular pathways mediating telomere maintenance and proposed that these mechanisms were selected because they generate heterogeneity. Similarly as noise in gene expression, stochastic events in telomeres may have an adaptive value allowing cells to sustain viable and flexible populations, with implications for fields ranging from evolutionary biology to molecular medicine (57).

Telomeric sequences in a complex with specific DNA-binding proteins also play a critical role in protecting cells from mistaking chromosome ends as broken DNA which can trigger unwanted repair and apoptotic responses. Here again, studies aimed at understanding of molecular mechanisms of stabilization of yeast mitochondrial telomeres were highly instrumental. Characterization of mitochondrial telomere-binding protein (mtTBP) in *C. parapsilosis* identified in collaboration with dr. Hiroshi Fukuhara (Curie Institute, Orsay, France) (59,60,29) yielded another example of evolutionary tinkering as a tool for solving problems associated with linearization of DNA genome (see also above).

Molecular architecture of yeast mitochondrial genomes

The complete DNA sequences of yeast mitochondrial genomes represent an excellent tool for molecular phylogenetics as well as a suitable platform for
comparative studies providing an insight into evolution of their molecular architecture and mechanisms governing the organellar inheritance (35). In 2004, we determined the complete sequence of the linear mtDNA of the yeast *C. parapsilosis* (38). Importantly, *C. parapsilosis* mitochondria harbor two types of DNA replicons; linear DNA molecules with the length 30,923 bp terminating with arrays of tandem repeats (i.e., nx 738 bp), which code for mitochondrial proteins, and extragenomic circular DNA molecules termed t-circles that are implicated in the dynamics of mitochondrial telomeres (see above). A survey of species closely related to *C. parapsilosis* revealed variant t-circles whose sequences correspond to modified arrays at the ends of the linear mtDNA. On the other hand, strains lacking the t-circles were shown to contain circularized genome derivatives formed by fusion of terminal regions of the linear form (14,48). This implies that the t-circle dependent pathway represents the main, or perhaps the only, mechanism for telomere replication operating in mitochondria of *C. parapsilosis* and its elimination results in defective telomere maintenance ultimately leading to circularization of the organellar genome.

In order to uncover molecular mechanisms leading to evolutionary emergence of the linear DNA genome occurring in *C. parapsilosis* mitochondria, we initiated a large scale comparative study of the mitochondrial genomes from diverse yeast species belonging mostly to the CUG clade. In collaboration with dr. Pfeiffer and dr. Kucsera (University of Szeged, Hungary), dr. Lang (University of Montreal, Canada), and dr. Wolfe (University of Dublin, Ireland) we determined mtDNA sequences of *C. frijolesensis*, *C. jiufengensis*, *C. metapsilosis*, *C. maltosa*, *C. neerlandica*, *C. orthopsilosis*, *C. salmanticensis*, *C. sojae*, *C. subhashii*, *C. vartiovaarae*, and *Magnusiomyces magnusii*. We uncovered surprising diversity of mitochondrial genome architecture among these species, ranging from circular to linear and multipartite linear genome form. Interestingly, the linear genomes differ in their telomeric structures (14,66; Valach, M., Farkas, Z., Fričová, D., Kucsera, J., Pfeiffer, I., Kosa, P., Lang, B.F., Griač, P., Tomáška, L., and Nosek, J., unpublished results).

Importantly, our studies of the mitochondrial genome architecture go beyond the field of organelle genetics and genomics and may shed some light on how linear chromosomes and the telomeres in general have evolved and are maintained (see above). At the same time, mtDNA sequences provide a basis for design of unique molecular markers applicable in clinical microbiology. Moreover, the studies of mtDNA architecture and replication may allow identification of specific molecular targets for possible therapeutic intervention against pathogenic yeasts (37,47).

**Structure and function of mitochondrial nucleoids**

The mtDNA molecules are packed into cytological structures termed nucleoids. These specific nucleo-protein complexes are fundamental segregating units of the organellar genome. Identification of the roles of individual nucleoid components and elucidation how their functions are orchestrated to ensure the mtDNA packaging, stability and segregation is crucial for understanding the molecular principles governing mitochondrial inheritance. Comparative analysis of the complete nuclear genome sequences from *S. cerevisiae*, *K. lactis*, *D.*
hansenii, C. albicans, C. parapsilosis, Y. lipolytica, and S. pombe revealed that each species contains essentially the same set of nucleoid proteins. This implies that, in spite of a significant degree of variability of their mitochondrial genomes, the nucleoid structure, mtDNA replication strategy, and possibly also the mode of mitochondrial inheritance are conserved among phylogenetically diverse taxa (41).

Interestingly, the least conserved components of the nucleoids are proteins known to interact directly with the mtDNA. In collaboration with laboratories of dr. Miyakawa (Yamaguchi University, Japan) and dr. Sedman (University of Tartu, Estonia), we identified that in C. albicans and C. parapsilosis, Abf2p, the principal DNA-packaging protein in mitochondria of S. cerevisiae, is replaced by structurally different HMG (high-mobility-group) box containing protein named Gcf1p (33,67). The functional analysis of the C. parapsilosis homolog together with systematic investigations of other nucleoid components provides an insight into mtDNA-packaging of topologically different chromosomal forms.

**Transport of adenine nucleotides across the mitochondrial inner membrane**

Mitochondria play a crucial role in energetics of eukaryotic cell. While most of the cellular ATP is generated in mitochondria by oxidative phosphorylation, most of ATP is consumed in cytosol. Transport of ADP and ATP - a substrate and product of oxidative phosphorylation - between mitochondria and cytosol is mediated by ADP/ATP carrier (AAC), an integral inner mitochondrial membrane protein. In yeast S. cerevisiae, three AAC isoforms (Aac1p, Aac2p and Aac3p) are encoded by three different genes, expression of which is regulated by environmental factors including oxygen and carbon. The major isoform Aac2p is the only one of three AACs that is essential for oxidative phosphorylation and is required for growth on respiratory media or for growth of ρ− cells. However, depending on genetic background, mutants with all AAC genes deleted may be viable in glucose media. As it is essential for cells to maintain ATP pool both in cytosol and in mitochondria, in cells lacking functional AAC other means of adenine nucleotide transport across inner mitochondrial membrane must exist. Sa1 protein, identified as a suppressor of lethality of deletion of AAC genes in certain yeast strains (Chen, Genetics 167, 607-617 (2004)), is a likely candidate for a mitochondrial net ATP importer. We have recently shown that Sa1 mediated ATP transport can be demonstrated in the conditions when transport by AAC is blocked either by mutation or by inhibitor (Laco *et al.* (2010) *FEMS Yeast Res.* (in press)).

**The role of mitochondria in the control of apoptosis**

Apoptosis in mammalian cells is regulated by proteins of Bcl-2 family, which govern the permeability of outer mitochondrial membrane for proteins including cytochrome c. While protein family includes pro-apoptotic members - Bax and Bak, which mediate the release of cytochrome c from mitochondria to the cytosol and thus initiate the apoptotic program, other members – Bcl-2 and Bcl-XL – inhibit their action to promote cell survival. Balanced activity of pro- and anti-apoptotic family members ensures cell survival in the absence of apoptotic stimuli. The subset of Bcl-2 family referred to as ‘BH3-only’ proteins constitutes an actual life/death switch. These proteins monitor a wide range of different insults and pro-death signals and in response to them activate Bax and Bak by a mechanism that is
still under dispute.

We have been using yeast *S. cerevisiae* as a model system, in which to study the mutual interactions of Bcl-2 proteins and Bcl-2 proteins with mitochondrial membranes. Although yeast do not have homologues of Bcl-2 family genes, ectopic expression of pro-apoptotic family members (Bax, Bak) induces the release of cytochrome c from mitochondria followed by cell death, both of which can be prevented by the coexpression of antiapoptotic family members (Bcl-XL, Bcl-2). Introduction of a third member – a “BH3-only” protein (e.g. Bim, Bik, tBid) – into yeast cells can repress the cell-rescuing ability of anti-apoptotic proteins (Drobcová, B., Polčic, P. *et al*., unpublished results). Current work in the lab is aimed at identifying components of mitochondrial membranes required for pore formation by Bcl-2 proteins as well as characterize interactions between propototic and antiapoptotic family members and mitochondrial membranes.

**Mitophagy: a selective degradation of mitochondria by autophagy**

Autophagy, a process involved in the degradation and the recycling of long-lived proteins and organelles to survive nitrogen starvation, is generally non-selective. However, recent data suggest that selective forms of autophagy exist that are able to specifically target several organelles including mitochondria (mitophagy).

In collaboration with dr. Manon and dr. Camougrand (University of Bordeaux 2, France), we obtained the first evidence that mitophagy is genetically controlled by *UTH1* gene. Uth1p is involved in longevity regulation and required for Bax activation of cell death in yeast. We demonstrated that Uth1p is required for removal of mitochondria into the vacuole during autophagy induced by rapamycin or nitrogen starvation in the presence of respiratory carbon source. Mitophagy inhibition in the Δuth1 strain occurs without diminishing the function of the autophagic machinery (3,10).

Rapamycin-induced autophagy is accompanied by the early production of ROS and by the early oxidation of mitochondrial lipid. Inhibition of these oxidative effects by resveratrol largely impaired autophagy of both cytosolic proteins and mitochondria, and delayed subsequent cell death. These results support a role of mitochondrial oxidation events in the activation of autophagy (12).

Yeast cells expressing mammalian Bax protein displayed autophagic features. However, the inactivation of autophagy did not prevent and actually slightly accelerated Bax-induced cell death, which suggests that autophagy might correspond to a survival attempt in apoptotic cells (11).

Electron microscopy investigations of mitochondrial autophagy in nitrogen-starved yeast cells grown in the presence of respiratory carbon source have established the existence of two distinct processes: the first one occurring very early, is selective for mitochondria and is dependent on the presence of the mitochondrial protein Uth1p; the second one occurring later, is not selective for mitochondria, is not dependent on Uth1p, and is a form of bulk autophagy (4,13).

Observations of yeast cells submitted to nitrogen starvation after growth on different carbon sources provided evidence that microautophagy, rather than macroautophagy, preferentially occurred in cells grown under non-fermentable conditions. Although mitophagy seems to be a morphologically different process from macroautophagy, this process still requires the participation of the main ‘macroautophagic’ genes (10,13).
Recently, by chemical and genetic manipulation of the intracellular level of glutathione in *S. cerevisiae*, we showed that mitophagy can be regulated independently of general autophagy, and that its implementation may depend on the cellular redox status (2,5).

**Relationships between regulation of ion homeostasis and dynamics of eukaryotic organelles**

Defects in the dynamics of cellular organelles often have fatal consequences documented by several examples of human diseases caused by deficiencies in cellular components involved in organelle biogenesis. Our research in this field is based on experimental results describing a selective effect of potassium ionophores, nigericin and valinomycin, on inner mitochondrial membrane of *S. cerevisiae* (Kováč et al., *Biochim. Biophys. Acta* 721, 341-348 (1982); Kováč et al., 1982, *Biochim. Biophys. Acta.* 721, 349-356 (1982); Kováč and Klobučníková, in: *Mitochondria*, edited by R.J. Schweyen et al.). To identify cellular components responsible for this phenomenon, we employed a genomic screen (“Tn-screen”) aimed at finding mutants resistant to the ionophores due to the insertion of a transposon into the corresponding locus (30). We found that in majority of the mutants the tolerance to ionophores was associated by changes on the level of mitochondria. For example, electron-microscopic analysis revealed that strains lacking *MDM31* or *MDM32* contain giant mitochondria and exhibit frequent loss of mitochondrial DNA. Importantly, we have found that in addition to the tolerance of *mdm31* and *mdm32* mutants to nigericin, they display decreased swelling ability of their mitochondria in isotonic solutions of potassium acetate (in spite of a presence of exogenous K⁺/H⁺ antiport) and mitochondrial morphology defects are suppressed by the addition of nigericin. These results indicated a close connection between regulation of ion homeostasis and mitochondrial morphogenesis. In the complementary genetic screen (“UV-screen”) we identified ionophore-resistant mutants of *S. cerevisiae* using UV mutagenesis (43). We isolated several valinomycin- and/or nigericin-resistant mutants exhibiting peculiar phenotypic characteristics. For example, one mutant was unable to grow on a complex media, whereas its growth on synthetic media was indistinguishable from the parental strain. The other mutant lost the ability to grow on complex media containing 0.2 M calcium cations, while Ca²⁺ at the same concentration did not inhibit growth of this strain on synthetic media. We reasoned that the inhibitory effect of complex media is caused by differences in composition of elements, as described recently by our laboratory (1). However, it is very likely that it represents a more complex phenomenon deserving a more detailed analysis. Another important observation resulting from our studies of the ionophore-resistant mutants (prepared by both Tn- and UV mutagenesis) is the apparent role of vacuoles in mediating the effects of the ionophores on the cell (43; Fričová, D., Bhatia-Kiššová, I., Kautmanová H., Tomáška, L., and Nosek, J., unpublished results). In summary, our results indicated a direct relationship between mitochondrial ion homeostasis, mitochondrial morphology a control of mitochondrial volume, lipid composition of cellular membranes and coordination of mitochondrial and vacuolar functions. This underlines the importance of the studies of the mechanisms mediating the effects of mitochondrial ionophores not only for the understanding of regulation of mitochondrial ion homeostasis, but also its connections to mechanisms involved in regulation of organelar dynamics and communication between membrane
compartments of the eukaryotic cell. We are currently continuing this research in collaboration with the laboratory of dr. Grič, dr. Klobučíková, and dr. Hapala (Institute of animal biochemistry and genetics, Slovak academy of sciences) employing a yeast deletion library for identification additional genes involved in mediating the effects of ionophores in yeast cells.

**Protein phosphorylation as means of signalling in yeast mitochondria**

Protein phosphorylation is one of the most frequent (if not the most frequent) types of post-translational modifications of proteins. For example, the genome of *S. cerevisiae* contains almost 150 protein kinase encoding genes and almost every third protein is phosphorylated on at least one position. However, there is only a limited number of characterized mitochondrial protein kinases. A computer-aided analysis revealed that less than 10 members of this large protein family are potentially localized in mitochondria (54). The low abundance of mitochondrially targeted protein kinases in yeast may reflect the reductive evolution of mitochondrial signaling components and/or the apparent lack of selection pressure for acquiring mitochondrially localized protein kinases encoded by the host genome. In collaboration with the laboratory of dr. H. Yde Steensma (Leiden University, The Netherlands), we have investigated the role of two putative protein kinases encoded by YIL042c (*PKP1*) and YGL059w (*PKP2*), with high sequence similarity to human pyruvate dehydrogenase kinases. We have demonstrated that the proteins localize in discrete submitochondrial regions, exhibit protein kinase activity *in vitro* and that they indeed regulate the activity of the pyruvate dehydrogenase complex (50). Aberrant pyruvate dehydrogenase kinase activities have been shown to participate in various metabolic disorders. Our studies on Pkp1 and Pkp2 thus provided further support for using yeast as models of human diseases (6).

**Yeasts as models for eukaryotic cell morphogenesis**

Yeasts are non-motile organisms; yet the ability to actively search for and harvest nutrients and avoid detrimental substances, respectively, is critical for their survival in nature. In addition, during the last few decades, changes in medical practice and in diseases to which humans are exposed have resulted in a significant increase in the importance of fungal infections in particular caused by opportunistic pathogens. Most of these microbial pathogens alternate between two forms; single oval (yeast) cells and multicellular filaments. The dimorphic growth plays an important role in environment exploration as well as during infection into human tissues. In addition to the yeast-filament transitions, *C. albicans* is capable of undergoing a different type of morphological change that has been termed ‘phenotypic switching’, an apparently powerful strategy how to come around the host's immune defense system. We employ several yeast species as models for investigation of molecular basis of dimorphism. In *C. parapsilosis* we have prepared molecular tools for genetic transformation of *C. parapsilosis* (15,36,68) and initiated several projects aimed at understanding mechanisms involved in regulation of cell morphogenesis (42). In *Y. lipolytica*, partly in collaboration with dr. Gaillardin’s laboratory (INRA-CNRS Paris-Grignon, France) we described relationships between nitrogen metabolism and regulation of dimorphic switch (7,52). We also initiated a systematic analysis of genes involved in regulation of
dimorphism in *Y. lipolytica* and found that protein kinase Cla4p is essential for filament formation and invasive growth of (51). Finally, we are interested in various aspects of cell biology of *Magnusimyces* (*Dipodascus, Endomyces*) *magnusii*, yeast species with giant multinuclear cells. We prepared genetic transformation system and constructed plasmid vectors (28; Jakúbková, M., Bhatia-Kiššová, I., unpublished results) that open a venue for understanding the means of regulation of cell growth, cytokinesis and karyokinesis in this peculiar eukaryotic microorganism.

*Foundations of cognitive biology*

Cognitive biology aims at a synthesis of data of various scientific disciplines within a single frame of conceiving life as epistemic unfolding of the universe (the epistemic principle). In accord with evolutionary epistemology, it considers biological evolution as a progressing process of accumulation of knowledge. The knowledge is embodied in constructions of organisms, and the structural complexity of those constructions which carry embodied knowledge corresponds to their epistemic complexity. In contrast to evolutionary epistemology, cognitive biology is based on the assumption that the molecular level is fundamental for cognition and adheres to a principle of minimal complexity, which stipulates that the most efficient way to study any trait of life is by studying it at the simplest level at which it occurs (16).

*Pedagogy as an experimental endeavor*

All research described above serves as means of education for students through the projects of their diploma and dissertation theses. In numerous cases our students co-authored a peer-reviewed paper before acquiring their M.Sc. degree. Importantly, we consider our pedagogical activities as part of our experimentation in a broader sense. Lectures and seminars provide unique opportunities to experiment with various teaching approaches and test them with the participating students (53,55). This, together with our research on yeasts, made our work in the last decade fun and helped us to overcome transient hurdles.

*List of relevant publications (2000-2009)*

55. Tomáška, L': Teaching how to prepare a manuscript by means of re-writing published scientific papers. Genetics 175, 17-20 (2007).


**Financial support:**

The work has been generously supported by several foreign and Slovak grant agencies. Particularly, we wish to thank Howard Hughes Medical Institute, Fogarty International Center, UNESCO-ROSTE, NATO, Slovak Research and Development Agency (APVV), Slovak Grant Agencies VEGA and KEGA, University Complutense Madrid and Comenius University in Bratislava for their support throughout the years.
Fig.: Molecular architecture of the mitochondrial genome of the pathogenic yeast *Candida parapsilosis*. The mitochondrial genome is represented by linear DNA molecules with defined terminal structures. In addition, *C. parapsilosis* mitochondria contain extragenomic circular molecules derived from the telomeric sequence (termed t-circles) implicated in the maintenance of the linear genome form.
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Research field, aims and directions
Activity of strictly pathogenic and opportunistically pathogenic yeasts and filamentous fungi

Individual topics pursued in the Laboratory and principal results

1. Identification of fungi by of microscopy, culture, pathogenicity, antigenic and allergenic structure
2. Proof of specific antigens, allergens, toxins, antibodies by means of different serological tests
3. Laboratory diagnostics of tropical mycoses
4. Proof of farmer’s lung and allergic alveolitis evoked by fungi
5. Production of antigens and allergens by different methods
6. Proof of Crohn’s disease by Saccharomyces cerevisiae

List of relevant publications (2000-2009)