

UNIVERSITÄT GRAZ

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imbm

Institut für

Molekularbiologie, Biochemie und Mikrobiologie

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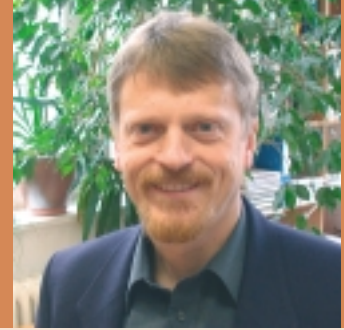
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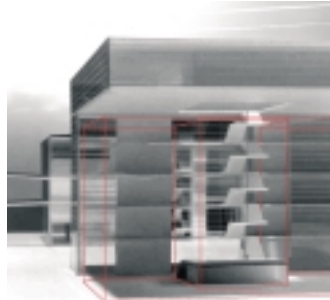
Report from the Chairman. 2002 was a remarkable year in the institute's history! Two new professors, appointed in December 2001, established their laboratories with a focus on yeast cell biology. Two new undergraduate curricula for Biochemistry and Molecular Microbiology, introduced in the Fall semester 2001/02, found increasing interest among students. Major investments in state of the art equipment greatly extended the scientific capabilities of the institute. Moreover, in November we celebrated the acquisition of one of four highly competitive and well-funded federal grants sponsored by the Austrian Genomics Initiative, GEN-AU.

Excellence in Research and Education. IMBM is committed to internationally competitive research and research-guided education. Financial support for our research comes from a high number of competitive grants funded by the FWF and other national agencies. Additionally, three European Union-funded projects are conducted at IMBM. Among them are projects dealing with antibiotic resistance transmission in bacteria, bacterial secretion mechanisms, biofilms, and the status and metabolism of vitamins in non-pathological human ageing.

A major long-term research initiative "SFB-Biomembranes" funded by the Austrian FWF (1995-2005) is focused on lipids and biomembranes. "G.O.L.D. – Genomics of Lipid-associated Disorders" is a major research initiative launched by the government in November 2002 in the framework of the genomics program, GEN-AU. The GOLD project is funded through 2005 with the option of a competitive renewal. Members of the institute participate in the K+ Centre of Excellence on Technical Biocatalysis, and engage in international exchange programs, e.g. Marie Curie Training Site, EU-INTAS with the New Independent States of the former Soviet Union, and numerous bilateral projects, underscoring the international networking of the institute and its scientists. The IMBM is firmly committed to research guided education. In 2002, over sixty diploma and Ph.D. students belonging to the molecular microbiology, biochemistry and chemistry curricula were involved in IMBM research programs.

Figure

Left: Building housing the Microbiology Division (Univ.-Platz 2)
Center: Main building of Karl-Franzens-University Graz
Right: Chairman, Sepp D. Kohlwein



Continuing development. In addition to existing staff members three professors will be appointed in 2003/04, with emphasis on molecular microbiology, cell biology and bioinformatics. At this point, the IMBM staff will include 6 professors, 11 associate and assistant professors, 15 technicians and administrative personnel. This expansion reflects the dynamic growth of the molecular life sciences, both on the research and educational levels. Currently, the institute is housed in three separate buildings scattered across the university campus. The existing physical limitations to further expansion will be overcome by the construction of a "Zentrum für Molekulare Biowissenschaften (ZMB)" – Center of Molecular Biosciences, that will also host a structural biology and bioinformatics unit. The building is scheduled to go into operation in 2005. In concert with the primary goal of enhancing the educational and research facilities the ZMB will provide space for start-up companies and will interact closely with industry. Continuing efforts to ensure the successful completion of the ZMB project, the current research program, and continuous growth in the number

of students enrolled in the molecular microbiology and biochemistry curricula, define a clear role for IMBM within the University of Graz, and the molecular life sciences in Graz.

Figure

Left: Part of the Biochemistry Division is housed in this building, Univ.-Platz 1/Schuberstr.1
 Center: Model of the planned "Center for Molecular Biosciences" (project Team Ohnmacht)
 Right: Biochemistry Division, housed at Heinrichstr. 31a

Institute Members

Scientific Staff – Biochemistry Division

Gregor Gorkiewicz, M.D., Research Associate
Günther Hämmerle, Ph.D., Research Associate
Sepp D. Kohlwein, Ph.D., Professor of Biochemistry, Chairman
Regina Leber, Ph.D., Assistant Professor
Oksana Tehlivets, Ph.D., Research Associate
Gerhard Nöhammer, Ph.D., Associate Professor
Karina Preiss-Landl, Ph.D., Assistant Professor
Gerald N. Rechberger, Ph.D., Research Associate
Harald Scholz, Ph.D., Research Associate
H. Manfred Tillian, Ph.D., Assistant Professor
Georg Wäg, Ph.D., Assistant Professor
Brigitte Winklhofer-Roob, M.D., Associate Professor
Heimo Wolinski, Ph.D., Research Associate
Rudolf Zechner, Ph.D., Professor of Biochemistry
Robert Zimmermann, Ph.D., Assistant Professor
Helmward Zollner, Ph.D., Associate Professor

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Scientific Staff – Microbiology Division

Helmut Bergler, Ph.D., Associate Professor
Kai-Uwe Fröhlich, Ph.D., Professor of Microbiology
Gregor Högenauer, Ph.D., Professor Emeritus
Helmut Jungwirth, Ph.D., Research Associate
Günther Koraimann, Ph.D., Associate Professor
Friederike Turnowsky, Ph.D., Associate Professor
Eva Wehrschütz-Sigl, Ph.D., Research Associate
Ellen Zechner, Ph.D., Associate Professor

Secretaries

Eveline Chatzatoglou-Hofer
Irmgard Jöstl (SFB-Biomembranes)
Michaela Majcenovic (SFB-Biomembranes)
Edith Sturmman
Sabine Voith

Technicians

Ing. Karin Bischof
Franz Dextl
Ing. Sandra Fuchsbichler (maternity leave)
Gabriela Gogg-Fassolter (maternity leave)
Ing. Susanne Häusler
Astrid Hermann
Mag. Birgit Juritsch
Ing. Monika Khabir
Astrid Knopf (apprentice)
Marianne Leitner
Theresa Maierhofer
Michaela Maritschnegg
Ing. Andreas Meinitzer
Yasmin Paar (apprentice)
Ing. Eva Pitters
Iris Prendler (apprentice)
Markus Reiterer
Regina Schreiner (maternity leave)
Renate Schreiber
Sandra Wuga (maternity leave)
Gertrude Zisser

IMBM Graduate Students

Mag. Vanessa Csitkovits
Mag. Gudrun Gann
Mag. Dijle Kipmen-Korgun
Mag. Christoph Magnes
Mag. Sonja Moser
DI Klaus Natter
Mag. Brigitte Pertschy
DI Andreas Reisner
Mag. Monika Riederer
Mag. Caroline Schober
Mag. Andrea Sovic
Isabella Sundl
Mag. Marisa Tschernatsch
Mag. Heidi Wieland
Mag. Doris Zahrl
Mag. Barbara Zavec

About the Institute

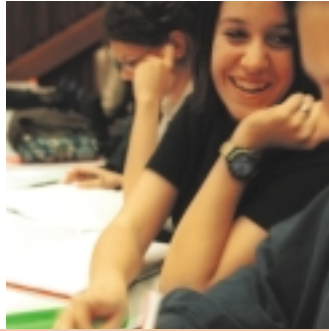


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In December 1999 the Institute of Microbiology merged with the Institute of Biochemistry with the aim to combine and strengthen both research- and teaching activities. As a result, the Institute of Molecular Biology, Biochemistry and Microbiology (IMBM) was created. Gregor Högenauer headed the institute until his retirement in September 2002. Sepp D. Kohlwein, appointed in 2001, is current chairman of the institute.

How we became IMBM ... In 1963, a Biochemistry Division was created within the Institute of Physical Chemistry. By 1968 Biochemistry was an independent institute under the direction of Erwin Schauenstein. Due to its significant growth, the newly formed Biochemistry Institute was relocated several times. After Schauenstein's retirement in 1988, Hermann Esterbauer became head of the department. Esterbauer was instrumental in establishing an internationally recognized research program focused on lipid peroxidation and the potential role of this process in lipid associated disorders, such as atherosclerosis. Independent project leaders on the assistant and associate professor level extended and enriched these research activities. The ongoing SFB- Biomembranes research project (1995-2005) was initiated under Esterbauer's leadership and he served as director of the project and head of the department until his untimely death in 1997. In 1998, Rudolf Zechner was appointed as Professor of Biochemistry. Zechner's research activities are focused on mammalian lipid and energy metabolism. The position left by Esterbauer was filled in 2001, through the appointment of Sepp D. Kohlwein, whose interest in lipid synthesis and membrane biogenesis is focused on fatty acid and neutral lipid metabolism in the yeast *Saccharomyces cerevisiae*.

The Institute of Microbiology was founded at the University of Graz in 1984. The first position of a Professor of Microbiology was filled through the successful recruitment of Gregor Högenauer, who at that time was section chief of the Experimental Chemotherapy Unit at the Sandoz Research Institute in Vienna. Högenauer brought a core of research staff and established research interests in the move from Sandoz to the university laboratories. Aspects of these research programs characterizing fatty acid biosynthesis and LPS biosynthesis in Gram-negative bacteria, as well as the genetics of conjugative antibiotic resistance factors in Gram-negative bacteria, were continued with the aim of defining potential drug targets for antimicrobial substances. Investigation of the mechanisms of action of inhibitory substances,



discovered at Sandoz, in model organisms of bacteria and fungi, have been pursued in detail. Development of the Institute's research program has been strongly shaped by independent investigators among the junior faculty. The number of positions for Associate Professors in the Microbiology Division has expanded to four. Growth of the institute was accompanied by installment of an elective undergraduate majors program in Microbiology followed by adoption of a regular degree program in Microbiology in 1992. Rapid increase in the number of students has far outpaced expansion of the scientific staff and laboratory and teaching space in the institute.

In 2000 a second full Professor of Microbiology, Kai-Uwe Fröhlich was appointed. Fröhlich's research activities, initiated during his tenure at the University of Tübingen, reinforce the institute's interest in AAA proteins and introduce the novel aspects of apoptosis and ageing in microorganisms.

Teaching

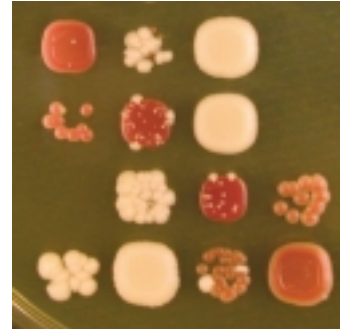


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Curricula at the IMBM. The IMBM is heavily involved in the microbiology/molecular biology and biochemistry curricula. In October 2001 the curricula within the biology program were renewed and four separate bachelor's and three master's degree programs were initiated. Also in 2001, a biochemistry master's program was launched within the chemistry curricula. IMBM offers a bachelor's program in molecular biology, lasting six semesters; a four-semester master's program in molecular microbiology; and a six-semester master's program in biochemistry and molecular biology. Many lectures, seminars and practical courses are supported by eLearning platforms, and further implementation of electronic media and web platforms for teaching is a high priority at IMBM. (1)

Bachelor's program "Molecular Biology": The laboratory and lecture courses of the first three semesters are shared by all four bachelor's programs in biology and cover the basics in chemistry, physics, mathematics, plant and animal biology, microbiology and genetics. In later semesters, specialization is achieved in theoretical and practical courses in molecular biology, microbiology, genetics, biochemistry, genetic engineering, and the application of modern information- and presentation-technologies. The bachelor's studies are concluded with two theoretical theses. Special emphasis in the program is given to practical training, to allow graduates to perform guided research in a wide range of fields, including applied pharmaceutical, medical, food and environmental research. Since it was first offered in 1994, the Diploma study of Microbiology became a highly popular subject in biology, attracting more than 100 new students each year. The number of applications to the new bachelor's program of Molecular Biology indicates that this popularity will continue.

Master's program "Molecular Microbiology": Building up on a bachelor's degree, students in Molecular Microbiology will take part in four semesters of specialized, research-guided training in microbiology, genetics and molecular biology, with strong emphasis on practical experience with state of the art techniques. The core program of required courses is supplemented with elective courses, which reflect the development of the field and the students' scientific interests. These include lectures and seminars for subjects such as molecular evolution, and extend to practical courses ranging from gene technology and gene expression, to genomics and proteomics. The master's theses usually require six months of independent work in the laboratory on a topic integrated in ongoing research projects.



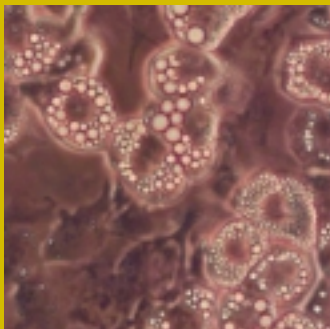
Master's program "Biochemistry and Molecular Biology": The master's program in Biochemistry and Molecular Biology (six semesters) builds on a four-semester general training in chemistry, physics and mathematics. The studies include lectures, seminars and lab courses on biochemistry, structural biology, analytics, microbiology, genetics, molecular and cell biology, gene technology, and bioinformatics. The master's thesis is completed with a six months independent project on a topic related to ongoing research projects.

Ph.D. programs: Fulfilment of master's degree requirements should prepare graduates for doctoral studies in a variety of programs including biochemistry, microbiology, molecular biology, pharmacology, bioorganic and technical chemistry, biomedical chemistry, or pathology. Many of the graduates of Microbiology or Biochemistry at the IMBM continue their training in a Ph.D. program or as postdocs at different Austrian and foreign research facilities. Others work in medical or food diagnostic facilities, biotechnology or pharmaceutical companies. So far, the market situation is positive. Moreover, the flexibility built into the curricula allows us to adjust the training to rapid developments in basic and applied research in academia and industry.

 [Link](#)

(1) <https://imbm-el.uni-graz.at>

Joint Research Programs at IMBM



Links

- (1) <http://www.gen-au.at/>
- (2) <http://Biomembranes.uni-graz.at>

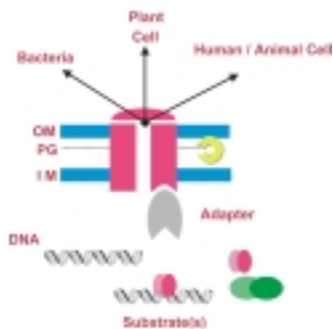
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Genomics of Lipid-Associated Disorders (G.O.L.D.). Dysregulation of the vascular and cellular metabolism of lipids and the excessive deposition of neutral lipids in adipocytes and the arterial wall are causally involved in the pathogenesis of obesity and atherosclerosis. The goal of this cluster-project, which started in November 2002, is to discover novel genes, processes and pathways that regulate lipid homeostasis in humans and mice. Additionally, the analyses will include yeast as a prototype model organism in lipid metabolism. The tremendous potential of high throughput genome- and proteome-based technologies and bioinformatics is utilized to analyze multi-organ expression profiles of mouse, and yeast mutants with specific defects in lipid metabolism. In such a discovery-driven, rather than hypothesis-driven, approach we expect to identify new genes involved in the regulation of cellular lipid homeostasis. The products of target genes will be characterized in their biochemical and physiological function, their molecular structure, and their potential role in the pathogenesis of disease.(1)

SFB-Biomembranes. The goal of the Spezial Forschungs Bereich (SFB)-Biomembranes is to make a major contribution to the understanding of the biosynthesis, function and dysfunction of lipids and biological membranes. Accordingly, the SFB-Biomembranes focuses a broad spectrum of thematic and technical expertise from various fields of the biosciences including genetics, biochemistry, molecular biology, cell biology, and electrophysiology and bioinformatics into a concerted research effort. Presently the project involves one coordination project and nine research projects covering the following four major themes: Lipid synthesis and membrane assembly, lipid transport, lipoprotein-membrane interactions, membrane function and dysfunction. The SFB-Biomembranes was started in 1995. It has gone through two rounds of re-evaluations (1998 and 2001) and is presently in its third funding period. It will end in September 2005.(2)

Marie Curie Training Site. The SFB-Biomembranes offers interdisciplinary doctoral training in bioinformatics, genetics, biochemistry, molecular biology, and biophysics. The trainees participate in current research activities to address questions related to lipid metabolism and membrane biology from diverse angles and benefit from the extensive scientific platform established within the SFB. IMBM contributes to the MC Training Site through projects utilizing biochemical and molecular assays, genetic engineering techniques, heterologous gene expression and high resolution microscopy (video, confocal), lipid analytics (nano-ESI MS/MS), microarray and proteome analyses.

EU projects within the Fifth Framework Program Quality of Life and Management of Human Resources



Links

(3) <http://www.clermont.inra.fr/vitage>

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Key Action 1: Food, Nutrition and Health. **Vitamin A, Vitamin E and Carotenoid Status and Metabolism during Ageing: Functional and Nutritional Consequences (VITAGE).**

Among the nutritional factors contributing to maintain health, fat-soluble vitamins (FSV) are crucial to protect against free radical-generated degenerative processes and impaired immune functions. The overall objective of the VITAGE project is to provide clear and sound scientific evidence of possible changes in status, metabolism and functions of the fat-soluble vitamins A and E, and of carotenoids during non-pathological ageing in humans. This goal will be achieved through a series of cross-sectional, single dose and long-term intervention studies including dietary depletion/repletion performed on 300 healthy male non-smoking volunteers 20 to 75 years of age from France (carotenoid intervention studies), Spain and Austria (vitamin E intervention studies). Biomarkers of exposure and variables related to status are measured both cross-sectionally under steady state conditions and longitudinally following intervention, along with immune and antioxidant functions. Metabolism is assessed in single-dose stable isotope tracer studies. Marketing opportunities for FSV-enriched dietetic foods specifically designed for the elderly are determined. The scientific

evidence obtained in this project will provide a basis for defining specific nutritional needs of the elderly and, perhaps, for developing a new sector of dietetic food products. (3)

Key Action 2: Control of Infectious Diseases. **Type IV secretion systems as targets for anti-infectious therapies (TFSS).**

Type IV secretion systems (TFSS) have been recently identified as being essential virulence determinants of many pathogenic bacteria of plants (e.g. *Agrobacterium tumefaciens*), animals (e.g. *Brucella suis*) and humans (e.g. *Helicobacter pylori*). A main effort of the EU consortium of leading scientists in the field of TFSS is to perform fundamental studies to better understand how TFSS contribute to pathogenicity. The assembly of the bacterial transporter in the bacterial cell envelope and the selection of macromolecules (DNA, proteins) that are translocated into the eukaryotic host cell are central issues in this project. Potential new drug targets will be identified and screened for inhibitors using high throughput screening (HTS). Identification of such inhibitory molecules specific for TFSS components are expected to lead to new therapies in the combat against infectious diseases.

Key Action 2: Control of Infectious Diseases. **Discovery of a New Class of Bioactive Compounds: Bacterial Conjugation Inhibitors (COINS).**

Combating bacteria with antibiotics directly cannot be effective in the long run because bacteria acquire antibiotic resistance (AR) genes easily from surrounding pools of resistant bacteria. Thus, an appropriate long

European Light Microscopy Initiative

– **ELMI**. The newly created ELMI establishes a unique communication network between European scientists working in the field of light microscopy and the manufacturers of these instruments.⁽⁵⁾ IMBM is established as an Austrian ELMI site, specializing in confocal laser scanning microscopy

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term public health objective would be to fight bacteria and the mechanisms of AR acquisition with a coordinated strategy. Acquisition of AR genes occurs primarily by the mechanism of bacterial conjugation. Preventing conjugation should markedly limit the dissemination of AR. This project's primary aim is to devise the most effective strategy to inhibit conjugation in or around the bacterial targets where AR is undesirable. For this purpose, we have isolated conjugation inhibitors through HTS of an extensive natural products library. The efficacy of these anti-conjugation products in controlled model environments will be analyzed and their molecular targets identified. Administration of a conjugation inhibitor should prevent AR acquisition.⁽⁴⁾

of living and fixed specimens, ranging from bacterial biofilms to yeast and mammalian tissue culture cells.

European Science Foundation.

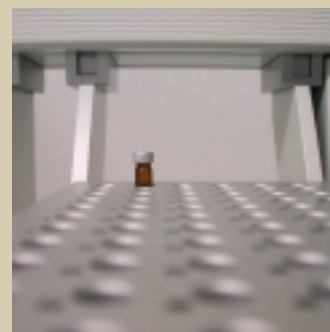
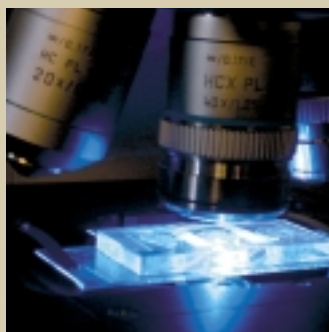
The IMBM is a coordinator for the project "Evolution and Population Genetics of Mobile Genetic Elements". Workshops funded by the ESF enable senior scientists as well as graduate students to participate in practical training courses and research programs linking molecular biologists, biochemists, microbial ecologists, physicians and bioinformatics specialists focused on bacterial genomes.⁽⁶⁾

Links

(4) <http://grupos.unican.es/genetica/coins.htm>

(5) <http://www.embl-heidelberg.de/elmi/>

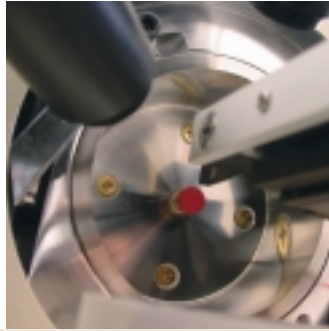
(6) <http://www.esf.org/workshops>



The excellent instrument base available at the IMBM supports both the research programs of the institute and the research-guided teaching programs in biochemistry and molecular microbiology. Additionally, several core facilities are established that offer central services for the lab members and outside investigators.

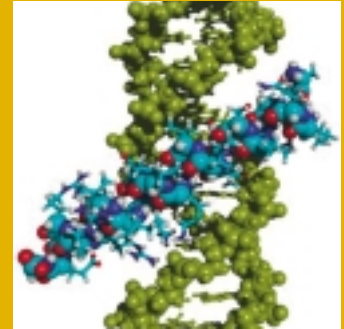
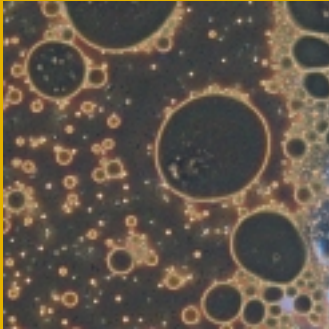
Mass spectrometry unit. The IMBM hosts a mass spectrometry facility specializing in lipid and protein analyses. A ThermoQuest Finnigan TSQ™ triple-quadrupole mass spectrometer with electrospray ionisation (ESI), nano-ESI and atmospheric pressure chemical ionisation (APCI) capabilities is predominantly used for the identification and characterization of lipid molecular species. Established methodologies and newly developed procedures provide important contributions to the current research projects on lipid synthesis, membrane trafficking, and pathological alterations resulting from mutations and disease. A ThermoQuest Finnigan LCQ™ Duo ion trap mass spectrometer capable of ESI, nano-ESI and APCI modes in conjunction with conventional or nano-HPLC is used for protein identification and the characterization of post-translational protein modifications. Finally, an AUTOMASS™ gas chromatography/MS system is used for the qualitative and quantitative analysis of small organic molecules.

Proteomics and Genomics Facilities. The IMBM is well equipped for genome-wide analyses at the mRNA and at the protein levels. Expression profiling in both yeast and mouse models is established at the institute as a standard technique in our genomics research. Special emphasis lies on characterizing the differentiation program during adipogenesis and on identifying expression changes resulting from altered lipid metabolism and stress response. Focused mouse cDNA arrays are produced and analysed in close collaboration within the SFB-Biomembranes and G.O.L.D. consortia and the bioinformatics facilities at the Technical University Graz. The proteomics facility includes several two-dimensional polyacrylamide gel electrophoresis units, calibrated densitometers and state of the art analysis software and is mainly used to study proteins involved in lipid metabolism and the development of metabolic diseases such as obesity, atherosclerosis or diabetes. The facility is operated in close collaboration with the mass spectrometry unit in house and allows the separation, identification and sequencing of proteins from complex mixtures.



Electronic light microscopy. The institute hosts two confocal laser scanning microscopes (Leica TCS4d and Leica SP2 AOBS two photon), micromanipulator/microinjection systems, and several video and CCD imaging systems which in conjunction with powerful image reconstruction software on Linux workstations allow animated 3D reconstruction of cells as small as yeast. This infrastructure offers excellent opportunities to analyze the spatial organization and dynamics of organelles and proteins in living cells. The main focus of the cytological studies is on yeast live cell microscopy, protein localization studies in yeast cells and mouse tissues, and *in situ* analysis of bacterial conjugation in biofilms. Additionally, microphotometry, a combination of UV-VIS-spectrophotometry and microscopy, is available at the institute for the quantification of substances in single cells.

Animal Health Service Laboratory. In collaboration with the Department of Veterinary Administration of the Styrian County Government (HR. Prof. Dr. Köfer), IMBM harbours a laboratory specializing in molecular diagnostic tests for animal health and food hygiene. This facility enables the rapid detection of zoonotic pathogens (e.g. *Salmonella*, *Campylobacter*) from various food products through the application of highly sensitive PCR and ELISA techniques. As part of a Styrian surveillance network, which monitors the development of antibiotic resistance in bacteria of animal origin, this laboratory offers several molecular assays for the detection and analysis of genetic resistance traits (e.g. glycopeptide resistance in enterococci). Furthermore, the analysis of residual chemotherapeutic agents in meat products and genetic testing of food animals completes the range of services offered.



Bacterial Mobile Genetic Elements

Gregor Gorkiewicz, M.D., Campylobacter research group
Günther Koraimann, Ph.D., Structure and Function of DNA transfer proteins
Ellen L. Zechner, Ph.D., Type IV Secretion Systems

Drug Resistance, Stress Response and Apoptosis

Kai-Uwe Fröhlich, Ph.D., Apoptosis in yeast
Gregor Högenauer, Ph.D., and **Helmut Bergler, Ph.D.**, Diazaborine resistance in yeast
Gerhard Nöhhammer, Ph.D., Quantitative microphotometry and histochemistry

Lipid Metabolism

Sepp D. Kohlwein, Ph.D., Lipid synthesis and membrane assembly in yeast
Friederike Turnowsky, Ph.D., Squalene epoxidase of yeast
Rudolf Zechner, Ph.D., Biochemistry and molecular biology of lipid and energy metabolism
Brigitte Winklhofer-Roob, M.D., Role of fat-soluble vitamins, fatty acids and lipid peroxidation in human nutrition

Figure

Left: Isolated lipid droplets from cultured fat cells.

Center: Lipid and energy metabolism in mouse models is a central topic of research at IMBM.

Right: Computer model of the N-terminal DNA binding helices of protein TraM bound to their DNA target sequence.

Bacterial Mobile Genetic Elements



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Bacterial cells use secretion processes to deliver protein and DNA molecules to other bacterial, plant or animal cells. Uptake of these secreted substances is often closely linked to infection and disease through direct and indirect effects unleashed in the recipient organisms (and their hosts). The research activities of three groups at the IMBM are dedicated to understanding this type of chemical cell to cell communication practiced by bacteria. A particular class of secretion systems, the Type IV group, are specialized in transmitting DNA from cell to cell. The consequences of uptake of genetic material, which can be stably maintained in the recipient cell and inherited by its progeny, can have very long term effects. The wealth of information provided by bacterial genome sequencing indicates clearly that processes of horizontal gene transfer, such as this, contribute profoundly to genetic diversity and evolution in bacteria. Vehicles of this genome plasticity, the mobile genetic elements (MGE), include plasmids, phage, transposons, pathogenicity islands, and many others. MGE are neither genome- nor species-specific, but may be exchanged widely among a broad spectrum of bacteria.

Modulation of the genomic composition determines both bacterial diversity and adaptability. The broad spectrum of physiological and virulence properties of bacterial pathogens reflects the existence of different subsets of genes carried by individual organisms. MGE are an important reservoir and motor of this variability. Antibiotic resistance genes, certain toxins, a number of virulence determinants and pathways for degradation of man-made pollutants are specifically located on MGE. Deeper understanding of the mechanisms involved in genome plasticity and in the evolution of bacterial species and variants is crucial to combating pathogenicity and to exploiting the opportunities presented by the flexible genome.

The IMBM research program aims to improve our understanding of the genetic, physiological, and clinical consequences of horizontal gene transfer, particularly those arising through Type IV secretion systems. Unravelling the molecular mechanisms involved elucidates fascinating aspects of cell – cell interactions and gene regulation networks, while providing us with an important rational framework for developing strategies to inhibit these transmission activities when linked to pathogenicity, surface adherence and antibiotic resistance spread.

Figure

Left: Electron micrograph of mating *E. coli* cells attached via an IncF pilus.
Center: Surface attached *E. coli* biofilm promoted by IncI conjugation system.
Right: Zones of mating cells transferring IncW plasmids (right).



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Our research is focused on the Gram-negative bacterium *Campylobacter*, a mucosal pathogen of significance to both humans and animals. The most prominent member, *Campylobacter jejuni*, is the world's major agent of human bacterial diarrhea. Furthermore, *C. jejuni* infection is the most common trigger of life-threatening Guillain-Barré syndrome, a disease presenting as acute neuromuscular paralysis. Despite improved understanding of *Campylobacter* virulence many questions regarding the distinct pathogenicity mechanisms of *Campylobacter* remain unanswered. In an attempt to gain more insight into the pathogen's virulence and to identify yet unknown virulence genes, we compared the genomes of the two *Campylobacter* subspecies, *C. fetus fetus* and *C. fetus venerealis*. Since these two pathogens are genetically highly related, yet exhibit substantial differences in virulence and host adaptation, we hypothesized that divergent genomic segments account for their host and tissue specificity. This approach led to the identification of several virulence genes, including a type IV secretion system (T4SS) exclusively present on a pathogenicity island of *C. fetus venerealis*. T4SS are known to play a key role in infection processes of various Gram-negative bacteria. Since these macromolecular transporters are present on the bacterial surface, they interact with host tissue, export toxins or deliver signaling molecules to establish infection. Through the use of knock-out mutants lacking T4SS genes and their use in *in vitro* infection assays we aim to refine our understanding of the T4SS mediated infection process and *Campylobacter* virulence.

Selected Publications

- Gorkiewicz G, Feierl G, Zechner R, and Zechner EL. Transmission of *Campylobacter hyointestinalis* from a pig to a human. *J. Clin. Microbiol.* 40:2601-2605, 2002.
- Krause R, Ramschak-Schwarzer S, Gorkiewicz G, Schnedl WJ, Feierl G, Wenisch C, and Reisinger EC. Recurrent septicemia due to *Campylobacter fetus* and *Campylobacter lari* in an immunocompetent patient. *Infection* 30:171-174, 2002.

Additional activities

- Development of a molecular surveillance program for diagnosis of zoonotic pathogens and their antibiotic resistance in food production, in collaboration with the Department of Veterinary Administration (Fachabteilung 8C) of the Styrian County Government.



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	Michael Manhart
	Anita Lassacher

Structure and Function of DNA Transfer Proteins. We study the DNA transfer mechanisms of the conjugative resistance plasmid R1. Plasmid R1 was isolated in the early 60's from *Salmonella typhi*, is approximately 100 kbp in size, encodes resistance to ampicillin, kanamycin, chloramphenicol, streptomycin and sulfonamides and possesses a large DNA region (40 kbp) encoding the DNA transfer system (the *tra* genes). The *tra* genes are arranged in one block and are very similar to other plasmids belonging to the IncF group, like F or R100. Bacterial conjugation is a macromolecular transport system termed Type IV Secretion System or T4SS. By studying bacterial conjugation, important insights can be obtained to better understand the role of T4SS in plant, animal and human pathogens.

In our current work on plasmid R1 the aim is to identify and characterize the function(s) of important transfer genes by the use of combined genetic, molecular biological and biochemical approaches. Both structural and functional aspects are being investigated. At present the work includes characterization of TraM, a specific DNA binding protein essential for conjugative transfer, protein 19, a T4SS specific muramidase which locally disrupts the bacterial cell wall and TraD, a protein acting at the interface between the T4SS and the nucleoprotein complex termed the relaxosome.

Selected Publications

- Verdino, P., Keller, W., Strohmaier, H., Bischof, K., Lindner, H., and Koraimann, G. (1999) The essential transfer protein TraM binds to the DNA as a tetramer *J Biol Chem* 274, 37421-37428.
- Bayer, M., Bischof, K., Noiges, R., and Koraimann, G. (2000) Subcellular localization and processing of the lytic transglycosylase of the conjugative plasmid R1 *FEBS Lett* 466, 389-393.
- Stockner, T., Plugariu, C., Koraimann, G., Högenauer, G., Bermel, W., Prytulla, S., and Sterk, H. (2001) Solution structure of the DNA-binding domain of TraM *Biochemistry* 40, 3370-3377.
- Bayer, M., Iberer, R., Bischof, K., Rassi, E., Stabenheiner, E., Zellnig, G., and Koraimann, G. (2001) Functional and mutational analysis of P19, a DNA transfer protein with muramidase activity *J Bacteriol* 183, 3176-3183.

Additional Activities

- Board member of the ÖGGGT (Sektionsleiter Graz, Österreichische Gesellschaft für Genetik und Gentechnik)

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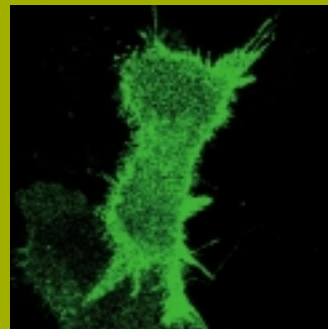
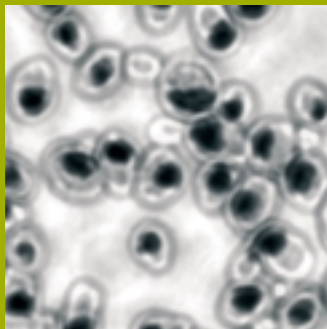
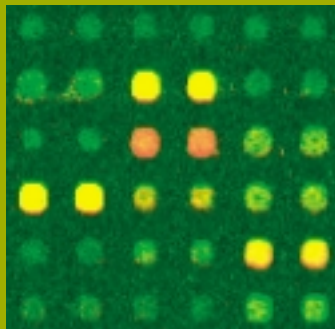
Ellen L. Zechner, Ph.D.
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Research in my group addresses the molecular mechanisms of Type IV secretion systems contributing to bacterial virulence and the cell to cell transmission process known as bacterial conjugation. Bacteria in nature adhere to surfaces and encase themselves in a hydrated matrix of polymers producing a slimy layer known as a biofilm. Organisms living in a differentiated biofilm community are resistant to antibiotics and difficult to remove or control. My group investigates the contribution of conjugation systems to the development of biofilms and the activity of secretion systems in surface-growing bacterial communities. These studies in situ involve fluorescent reporter systems combined with confocal and epifluorescence microscopy to visualize individual cells and track the secretion events that transmit DNA from one cell to another. The molecular mechanisms of the conjugal secretion process is also studied using biochemical and molecular genetic approaches to gain insights to regulation of the initial cell contact, enzymatic processing of DNA molecules destined for transfer, and recognition of correct export molecules by the multiprotein transport machinery. A third area of activity is dedicated to evaluating the contribution of newly discovered secretion systems to the host and tissue specificities of pathogenic bacteria. Finally the insights we gain into how these related systems contribute to the ability of bacteria to adhere and differentiate, to communicate, to transfer genes including those that confer resistance to antibiotics, and to behave as pathogens are complemented by an active interest in discovery and development of inhibitory substances that disrupt these processes with a long term goal of intervention. (1)

Selected Publications

- Karl, W., Bamberger, M. and E. L. Zechner (2001). Transfer Protein TraY of Plasmid R1 Stimulates TraI-Catalyzed *oriT* Cleavage In Vivo, *J. Bacteriol.* 183: 909-914.
- Schröder, G., Krause, S., Zechner E.L., Traxler, B., Yeo, H.-J., Lurz, R., Waksman, G., and E. Lanka (2002). TraG-like Proteins of DNA Transfer Systems and of the *Helicobacter pylori* Type IV Secretion System: the Inner Membrane Gate for Exported Substrates? *J. Bacteriol.* 184: 2767-2779. (This paper was featured under "Journal Highlights" (2002) *ASM News* 68: 345.)
- Reisner, A., Molin, S. and E. L. Zechner (2002). Recombinogenic engineering of conjugative plasmids with fluorescent marker cassettes, *FEMS Microbiol. Ecol.* 42: 251-259.
- Reisner, A., Haagensen, J. A., Schembri, M. A., Zechner, E. L. and S. Molin (2003). Development and maturation of *Escherichia coli* K-12 biofilms, *Mol. Microbiol.* In press.

Drug Resistance, Stress Response and Apoptosis



20

In their natural environment, cells are exposed to various forms of stress, e.g., toxic substances, reactive oxygen species, or irradiation. Cellular reactions to stress range from detoxification and toxin removal, to repair of cell damage, to induction of programmed cell death. The amount of stress and the cellular response it invokes have a crucial influence on the well-being and life span of the organism.

Different mechanisms have evolved by which cells can acquire resistance against toxic substances. One important mechanism of resistance is due to the alteration of transcription factors, leading to increased expression of their target genes. These targets can be, for instance, a number of drug efflux pumps with broad substrate specificity located in the plasma membrane. Consequently, increased expression of these pumps results in cross-resistance to a large number of unrelated compounds. Transcription factors involved in drug resistance are embedded in complex regulatory networks that often overlap with cellular stress responses, including oxidative stress.

Oxidative stress causes oscillating changes of thiol-disulfide equilibria in cells and cellular proteins, resulting in the generation of reactive sulfur species (RSS). RSS occur in malignant tumors, but also in normal cells spatially distant to the tumor; thiol-disulfide equilibria also significantly respond to environmental changes. Analysis of the protein thiol and disulfide content by microphotometry allows investigation of different forms of stress at the level of single cells. Cytophotometric quantification of both DNA and protein enables cell type-analysis as well as the recognition of apoptosis.

Apoptosis is a cellular suicide program which can be triggered by oxygen stress or irreparable DNA damage. In multicellular animals (like humans), it works beneficially to protect the organism from cancer and viral infections and it is essential for normal development. Apoptotic yeast is a useful tool to identify components of the basic apoptotic apparatus and to screen for drugs stimulating or inhibiting the death process. The availability of several thousands of knock out strains and the potential for genetic manipulations makes the yeast *Saccharomyces cerevisiae* an excellent eukaryotic model system for the analysis of complex regulatory processes like stress response and apoptosis.

Figure

Left: DNA array example showing differentially expressed genes in diazaborine-resistant yeast mutants.
Center: Apoptotic markers of yeast. TUNEL staining showing DNA fragmentation.
Right: Stressed tissue cell expressing a GFP-tagged protein at the cell surface.

Link

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I am interested in the common functions and phylogenetic relationship of AAA proteins. AAA proteins are ATPases involved in various essential cellular pathways, like vesicular transport, cytosolic proteolysis and ubiquitination, the cell division cycle and meiosis. I have set up a WWW-accessible database as the international information center for AAA proteins (1).

Among the AAA proteins, we focus on Cdc48 protein, which is highly conserved between yeast and man (>70% sequence identity), and participates in vesicle fusion and in the extraction of damaged proteins from the ER. The phenotype of a point mutation of Cdc48p shifted my interest to apoptotic phenomena in unicellular organisms. The mutant performs cell death with characteristic markers of apoptosis. Our group found that many inducers of animal apoptosis also function in yeast. Natural scenarios resulting in apoptotic death of yeast cells are cellular ageing (both replicative and chronological ageing) and various forms of environmental stress (oxidative stress, acidic stress, exposure to radiation). We have identified the yeast orthologue of the key executors of mammalian apoptosis, the caspases. We plan to use yeast to get a complete picture of the evolution and basic machinery of apoptosis. We are investigating the physiological role for apoptosis in yeast. Our main focus is on the connection of apoptosis and cell ageing, and the role of ROS (reactive oxygen species) in the death process.

Selected Publications

- Laun, P., A. Pichova, F. Madeo, J. Fuchs, A. Ellinger, S. Kohlwein, I. Dawes, K.-U. Fröhlich und M. Breitenbach (2001). Aged mother cells of *S. cerevisiae* show markers of oxidative stress and apoptosis. *Mol. Microbiol.* 39: 1166-1173.
- Fröhlich, K.-U. (2001). An AAA family tree. *J. Cell Sci.* 114: 1601-1602
- Ligr, M., I. Velten, E. Fröhlich, F. Madeo, M. Ledig, K.-U. Fröhlich, D. H. Wolf und W. Hilt (2001). The proteasomal substrate *Stm1* participates in apoptosis-like cell death in yeast. *Mol. Biol. Cell* 12: 2422-2432.
- Rabinovich, E., A. Kerem, K.-U. Fröhlich, N. Diamant, and S. Bar-Nun (2002). AAA-ATPase p97/Cdc48p, a Cytosolic Chaperone Required for Endoplasmic Reticulum-Associated Protein Degradation. *Mol. Cell Biol.* 22: 626-634.
- Madeo, F., E. Herker, C. Maldener, S. Wissing, S. Lächelt, M. Herlan, M. Fehr, K. Lauber, S. J. Sigrist, S. Wesselborg, und K.-U. Fröhlich (2002). A Caspase-Related Protease Regulates Apoptosis in Yeast. *Mol. Cell* 9: 911-917



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Diazaborine resistance in *Saccharomyces cerevisiae*. We are interested in the molecular mechanisms leading to diazaborine resistance in microorganisms. Diazaborine is a potent inhibitor of fatty acid biosynthesis in Gram-negative bacteria through interference with the enzymatic activity of the enoyl-ACP reductase FabI. We found that diazaborine also inhibits growth of the eukaryotic organism *Saccharomyces cerevisiae*. In this organism diazaborine interferes with RNA metabolism. Resistance to the inhibitor is caused by two independent mechanisms. One resistance mechanism involves allelic forms of the genes *PDR1*, *PDR3* and *YAP1*. These genes encode transcription factors which cause overexpression of membrane located efflux pumps. Overexpression of the transporters reduces the effective intracellular concentration of diazaborine by pumping it into the surrounding medium or into the vacuole.

The other resistance mechanism is specific for diazaborine and is connected with allelic forms of gene *DRG1*. This gene encodes an AAA-(ATPases Associated with a variety of cellular Activities) family member with unknown function. The main goal of our current research is to identify the mode of action of diazaborine in *S. cerevisiae* and to understand how protein Drg1 can render yeast cells resistant to this inhibitor.

Selected Publications

- H. Bergler, P. Wallner, A. Ebeling, B. Leitinger, S. Fuchsbichler, H. Aschauer, G. Kollenz, G. Högenauer, and F. Turnowsky. 1994. Protein EnvM is the NADH dependent enoyl-ACP reductase (FabI) of *Escherichia coli*. J. Biol. Chem. 269: 5493-5496.
- F. Wendler, H. Bergler, K. Prutej, H. Jungwirth, G. Zisser, K. Kuchler, and G. Högenauer. 1997. Diazaborine resistance in the yeast *Saccharomyces cerevisiae* reveals a link between *YAP1* and the pleiotropic drug resistance genes *PDR1* and *PDR3*. J. Biol. Chem. 272: 27091-27098.
- H. Jungwirth, F. Wendler, B. Platzer, H. Bergler, and G. Högenauer. 2000. Diazaborine resistance in yeast involves the efflux pumps Ycf1p and Flr1p and is enhanced by a gain of function allele of gene *YAP1* Eur. J. Biochem. 267: 4809-4816.
- H. Jungwirth, H. Bergler and G. Högenauer. 2001 Diazaborine treatment of baker's yeast results in stabilization of aberrant mRNAs. J. Biol. Chem. 276: 36419-36424
- A. Zakalskiy, G. Högenauer, T. Ishikawa, E. Wehrschütz-Sigl, F. Wendler, D. Teis, G. Zisser, A. C. Steven and H. Bergler. 2002 Structural and Enzymatic Properties of the AAA-protein Drg1p from *Saccharomyces cerevisiae*: Decoupling of Intracellular Function from ATPase Activity and Hexamerization. J. Biol. Chem. 277: 26788-26795.



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23

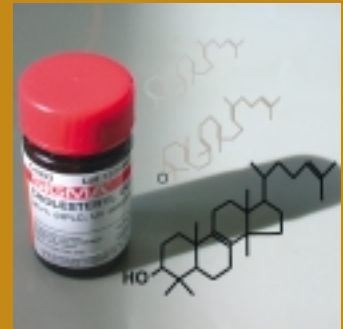
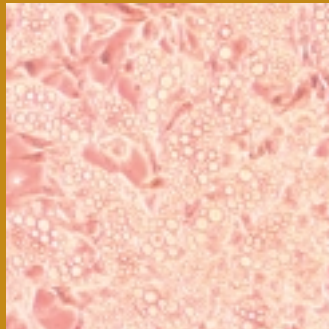
Microphotometry (MP), the combination of UV-VIS-spectrophotometry and microscopy, is an analytical method for the quantification of substances located within single cells or in the extracellular matrix. The limit of detection is 10^{-17} moles/ μm^2 .

One of our goals is the optimization and quantification of histochemical methods to facilitate cancer diagnosis based on tumor-associated changes of protein thiols and disulfides in cells and tissue. These changes may serve as biomarkers of oxidative stress in normal tissue in the neighbourhood of malignant tumors (field effect) but also in normal skin distant to tumors (extended field effect). For instance, tumor-associated oxidative modifications of nuclear protein disulfides can be monitored in buccal cells that are easily extractable from the human oral cavity, providing a useful marker for tumor diagnosis. In addition, protein-thiol-disulfide equilibria respond to environmental conditions, and are affected by short-term (range of weeks) and long-term changes of the weather. Our data collected over several years indicate that thiol-disulfide-equilibria may be used as biosensors for global climate change and, surprisingly, also for extended weather prognosis. Novel DNA-protein-double staining methods developed in our lab allow microphotometric quantification of both DNA and nuclear protein in single cells, providing insight into cell cycle status and the regulation of their synthesis and degradation during cellular growth and differentiation.

Selected Publications

- Nöhhammer,G., and Desoye,G. Mercurochrome can be used for the histochemical demonstration and microphotometric quantitation of both protein thiols and protein (mixed) disulfides. *Histochem Cell Biol.* 107: 383-390, 1997.
- Mandl,M., Haas,J., Bischof, P., Nöhhammer,G., and Desoye,G. Serum-dependent effects of IGF-I and insulin on proliferation and invasion of human first trimester trophoblast cell models. *Histochem Cell Biol.* 117: 391-399, 2002.
- Nöhhammer,G. Cytophotometric investigations on oscillating thiol-disulfide equilibria and oxidized protein sulfur. In: *Thiol metabolism and redox regulation of cellular functions*, Pompella,A., Banhegyi, G., and Wellman-Rousseau, M., Eds., IOS-Press, Amsterdam, pp. 48-60, 2002.

Lipid Metabolism



24

Lipids are essential in all living organisms because of their pivotal role in the formation of biological membranes. Additionally, lipids are important energy substrates and participate in various cell signaling processes. Over the last four decades, an increasing number of research groups from various faculties and universities in Graz has focused their scientific interest on lipids. Through many joint programs and collaborative projects, Graz has developed into a "center of lipidology". Research groups of the IMBM are substantially contributing to this development with projects that span the field of lipid biology from yeast to man.

The yeast *Saccharomyces cerevisiae* provides a well established model system for lipid and biomembrane research. Accordingly, several research groups are involved in the identification and characterization of factors involved in synthesis and the catabolism of fatty acids, neutral lipids and sterols in yeast. Key enzymes in fatty acid and ergosterol synthesis such as acetyl-CoA carboxylase and squalene epoxidase, respectively, as well as lipases and hydrolases involved in lipid degradation are extensively studied.

Considering the important role of lipids in energy metabolism and their causal involvement in the pathogenesis of such prevalent diseases as obesity and atherosclerosis, the function of various genes are also studied in genetically modified mouse models. Transgenic and knock-out mouse lines that overexpress or lack genes for lipolytic enzymes and enzymes involved in the synthesis of neutral lipids are characterized.

Finally, several projects within the IMBM aim at the discovery of novel genes and processes that are involved in the lipid and lipoprotein metabolism of humans. Especially, the effects of lipid peroxidation and the role of antioxidants are investigated to elucidate their role in the development of cardiovascular diseases.

Figure

Left: 3D reconstruction of a yeast cell and subcellular structures imaged by confocal laser scanning microscopy.

Center: Lipid accumulation in cultured fat cells.

Right: Sterols are important risk factors in the development of coronary heart disease. Squalene epoxidase is a key enzyme in sterol synthesis and an important target for drugs.

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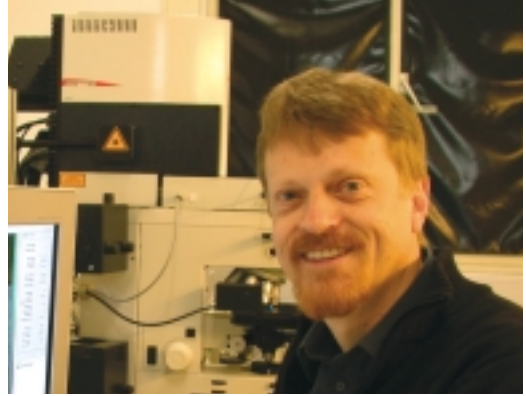
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🌐 Links

(1) <http://Biomembranes.uni-graz.at>

(2) <http://www.gen-au.at>

(3) <http://YPL.uni-graz.at>



Sepp Dieter Kohlwein, Ph.D.
Biochemistry Division

25

Lipid synthesis and membrane assembly in yeast. Our research interests focus on fatty acid and neutral lipid (triglyceride) metabolism in the yeast *Saccharomyces cerevisiae*. Special emphasis lies on problems related to lipid-associated disorders, making full use of the experimental potential of yeast as a model with high significance for biomedical research in humans. In the framework of SFB-Biomembranes (1) we study the regulation of fatty acid synthesis (acetyl-CoA carboxylase) and the regulatory role of fatty acids in cellular metabolism. Related to this problem is the question of fatty acid storage in the form of triglycerides, and their mobilization by lipases (2). These studies involve biochemical and analytical (nano electrospray-ionization tandem mass spectrometry), molecular, and cell biological techniques as well as genomics, and bioinformatics approaches. Cytological studies in yeast and other cell types involve video and confocal laser scanning microscopy. We have established a yeast protein localization database, YPL.db (3), that displays microscopic localization patterns of some 500 GFP-tagged proteins relevant to lipid metabolism. We also employ various yeast species as hosts for heterologous gene expression, for functional studies *in vivo* and large scale protein production.

Selected Publications

- Schneider, R., B.Brügger, R.Sandhoff, G.Zellnig, A.Leber, M.Lampl, K.Athenstaedt, C.Hrastnik, S.Eder, G.Daum, F.Paltauf, F.Wieland, and S.D.Kohlwein. 1999. Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS) Analysis of the Lipid Molecular Species Composition of Yeast Subcellular Membranes Reveals Acyl Chain-based Sorting/Remodeling of Distinct Molecular Species En Route to the Plasma Membrane. *J. Cell Biol.* 146 741-754.
- Kohlwein, S.D. 2000. The beauty of the yeast. *Light microscopy at the limits of optical resolution.* *Microsc. Res. Tech.* 51(6), 511-529.
- Kohlwein, S.D., S.Eder, C.-S.Oh, C.E. Martin, K.Gable, D.Bacikova, and T.Dunn. 2001. The *Saccharomyces cerevisiae* Tsc13p/YDL015c Protein is Required for Fatty Acid Elongation and Localizes to a Novel Structure at the Nuclear/Vacuolar Interface. *Mol. Cell. Biol.* 21, 109-125.
- Shirra, M.K., J. Patton-Vogt, A. Ulrich, O. Liuta-Tehlivets, S. D. Kohlwein, S. A. Henry and K. M. Arndt. 2001. Inhibition of acetyl-CoA carboxylase activity restores expression of the *INO1* gene in a *snf1* mutant strain of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 21(17): 5710-22.
- Habeler, G., K. Natter, G. G. Thallinger, M. Crawford, S. D. Kohlwein and Z. Trajanoski. 2001. YPL.db: The Yeast Protein Localization Database. *Nucl. Acids Res.* 30: 80-83.

Additional Activities

- Chairman of the IMBM
- Vice director of the SFB-Biomembranes
- Vice director of G.O.L.D. Genomics of Lipid-associated Disorders (GEN-AU Program)



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Squalene epoxidase of *Saccharomyces cerevisiae*. Ergosterol is an essential component of fungal membranes and, thus, inhibition of ergosterol biosynthesis leads to growth inhibition. One enzyme in the sterol biosynthesis pathway is squalene epoxidase which is the target for a group of potent antifungal compounds, the allylamines. One line of investigation is directed towards the elucidation of the enzymatic activity of squalene epoxidase (Erg1p) of *Saccharomyces cerevisiae* as our model organism, its purification and interaction with the inhibitor terbinafine and with predicted accessory proteins in the yeast cell. Squalene epoxidase variants from terbinafine resistant- and terbinafine supersensitive and temperature sensitive mutants will allow us to approach these questions. Inhibition of squalene epoxidase leads to ergosterol deficiency, followed by the induction of *ERG1* gene expression. We investigate the specific sequences in the *ERG1* promoter which are required for induced expression and identify the transcription factor(s) which are involved in the transcriptional regulation of *ERG1* gene expression. In addition, we use terbinafine resistant mutants to characterize the resistance mechanisms which lead to insensitivity against allylamines at the molecular level.

Selected Publications

- R. Leber, K.M. Landl, E. Zinser, H. Ahorn, A. Spök, S.D. Kohlwein, F. Turnowsky, and G. Daum. 1998. Dual localization of squalene epoxidase, Erg1p, in yeast reflects a relationship between the endoplasmic reticulum and lipid particles. *Mol. Biol. Cell* 9: 375-386.
- R. Leber, R. Zenz, K. Schröttner, S. Fuchsichler, B. Pühringer, and F. Turnowsky. 2001. A novel sequence element is involved in the transcriptional regulation of expression of the *ERG1* (squalene epoxidase) gene in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 268: 914-924.

Additional Activities

- Board member and speaker of the Federal Genetic Engineering Commission
- Delegate of the Austrian Society for Molecular Biology and Biochemistry in the „dialog<> gentechnik“ platform



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27

Biochemistry and Molecular Biology of Lipid and Energy Metabolism. The coordinated uptake of non-esterified fatty acids, lipids and lipoproteins in adipocytes, muscle cells, macrophages and other cell types is of central importance for both the metabolism of plasma lipoproteins and energy homeostasis in mammals and humans. Dysregulation of these processes is associated with such prevalent diseases as hyperlipidemias, atherosclerosis, diabetes mellitus, and obesity. The current research activities of this laboratory focus on the tissue-specific role of various lipases (lipoprotein lipase, endothelial lipase, hormone-sensitive lipase) for the metabolism of plasma lipoproteins and lipid metabolism in fat cells, myocytes, and macrophages. Transgenic and knock-out mouse lines that either overexpress or lack these enzymes are characterized in detail to elucidate the metabolic adaptations of variations in the cellular fatty acid, lipid and lipoprotein uptake. In addition we have started to identify and characterize new genes that are involved in lipid metabolism and energy homeostasis of mammals within the Austrian genome project GEN-AU.

Selected Publications

- S. Levak-Frank, W. Hofmann, P.H. Weinstock, H. Radner, W. Sattler, J.L. Breslow, R. Zechner. Induced mutant mouse lines that express lipoprotein lipase in cardiac muscle but not in skeletal muscle and adipose tissue have normal plasma triglyceride and HDL-cholesterol levels. *Proc. Natl. Acad. Sci. USA* 96, 165-3170 (1999).
- R. Zimmermann, U. Panzenböck, A. Wintersberger, W. Graier, O. Glatter, G. Fritz, G.M. Kostner, R. Zechner. Lipoprotein lipase mediates the uptake of glycated LDL in fibroblasts, endothelial cells and macrophages. *Diabetes* 50, 1643-1653 (2001).
- J. G. Strauss, S. Frank, D. Kratky, G.Hämmerle, A.Hrzenjak, Gabriele Knipping, A. von Eckardstein, G. M. Kostner R. Zechner. Adenovirus mediated rescue of lipoprotein lipase-deficient mice: Lipolysis of triglyceride-rich lipoproteins is essential for HDL maturation in mice. *J. Biol. Chem.* 276, 36083-36090 (2001)
- G. Hämmerle, R. Zimmermann, M. Hayn, C. Theussl, G. Waeg, E. Wagner, W. Sattler, T. Magin, E. Wagner, R. Zechner. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle and testis. *J. Biol. Chem.* 277, 4806-4815 (2002)
- G. Haemmerle, R. Zimmermann, J.G. Strauss, D. Kratky, M. Riederer, G. Knipping, R. Zechner. Hormone-sensitive lipase deficiency in mice causes changes in the plasma lipid profile by affecting the tissue-specific expression pattern of lipoprotein lipase in adipose tissue and muscle. *J. Biol. Chem.* 277, 12946-12952 (2002)

Additional Activities

- Director of G.O.L.D. Genomics of Lipid-associated Disorders (GEN-AU Program)
- Director of the SFB-Biomembranes



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- (1) <http://www-ang.kfunigraz.ac.at/~winklhof>
- (2) <http://www.clermont.inra.fr/vitage>

Role of fat-soluble vitamins, fatty acids and lipid peroxidation in human nutrition. The impact of nutrition on maintaining health is attracting increasing attention. High intake of fruits and vegetables rich in antioxidant vitamins and modulation of dietary fat intake play a major preventive role in the development of degenerative diseases. Our research activities focus on human nutrition and metabolism with special emphasis on fat-soluble vitamins, fatty acids and lipid peroxidation (1). A major goal is to help define optimal intake and status of antioxidant vitamins in health and disease and to provide evidence of how these can be achieved. Using stable isotope tracers, we currently investigate vitamin E metabolism in healthy volunteers and will continue to do so by testing the influence of specific drugs. Within the framework of the EU-funded RTD project VITAGE we concentrate on the impact of ageing on status, metabolism and functions of fat-soluble vitamins (2). Oxidant stress is not only involved in ageing but also plays a key role in different pathophysiological conditions. Our work in this area addresses the mechanisms of lipid, protein and DNA oxidation and possible protective effects of antioxidants in human intervention trials. A major goal is to develop new therapeutic strategies for conditions with a very high oxidant load such as exposure to transition metal ions and chronic inflammatory disorders. Because a gold standard of how to assess oxidant stress is not available, we evaluate biochemical and immunological methods for detecting lipid, protein and DNA oxidation and assessing antioxidant levels that exert efficient protection. Well-established local and international collaborations help achieve these ambitious goals.

Selected Publications:

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Campus Map

