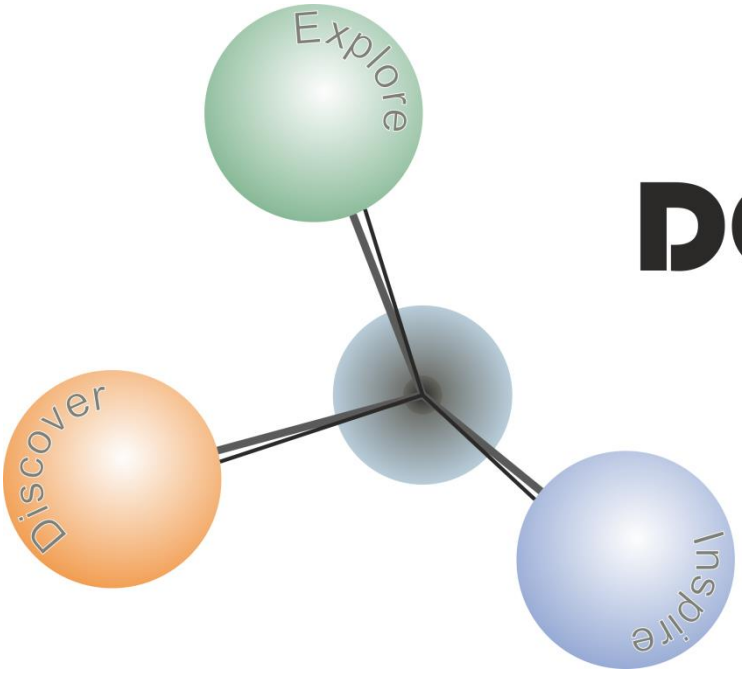


DOC DAY 17

JOINT SCIENTIFIC MEETING

OF THE NAWI GRAZ DOCTORAL SCHOOLS OF

MOLECULAR BIOSCIENCES AND BIOTECHNOLOGY

AND MOLECULAR BIOLOGY AND BIOCHEMISTRY

February 3rd 2017

17th DocDay

Friday, 3rd of February 2017
Kopernikusgasse 24, HS H
Graz University of Technology

Organizing Committee

Pia Benedikt, Dina Hofer, Katharina Huber,
Torben Kühnast and Lisa Katharina Maresch

Program

09:00 – 09:15	**Welcome and opening remarks** Vice-Rector of University of Graz **Ao.Univ.-Prof. Dr. Renate Dworczak** *Head of Doctoral School Molecular Biosciences and Biotechnology* **Assoc.-Prof. Dr. Juliane Bogner-Strauss** (Graz University of Technology)
	Session 1 Chair: Pia Benedikt, Lisa Katharina Maresch
09:15 – 10:00	**Plenary Lecture: Alexandros Vegiopoulos** **(German Cancer Research Center, Heidelberg, Germany)** *Metabolic stem cell responses and the plasticity of fat*

10:00 – 10:15	**Oral Presentation 1: Ursula Feiler** *Identification and characterization of cancer-associated cachexia inducing factors*
10:15 – 10:30	**Oral Presentation 2: Pia Benedikt** *UCP1 deficiency in cancer-associated cachexia*
10:30 – 10:45	**Oral Presentation 3: Xie Hao** *Molecular mechanism under browning in cancer-associated cachexia*
10:45 – 11:15	Coffee break
	Session 2 Chair: Katharina Huber, Pia Benedikt
11:15 – 12:00	**Plenary Lecture: Lorenzo Galluzzi** **(Weill Cornell Medical College, New York , US)** *Inside – Out: A story of intracellular stress and organismal homeostasis*
12:00 – 12:15	**Oral Presentation 4: Katharina Huber** *The role of N-acetyltransferase 8-like in energy metabolism and autophagy*
12:15 – 12:30	**Oral Presentation 5: Angelina Gross** *Acetyl-CoA Carboxylase as key regulator of autophagy in the yeast Saccharomyces cerevisiae*
12:30 – 12:45	**Oral Presentation 6: Andreas Aufschnaiter** *LRRK2 impairs autophagy and endocytic pathways in a yeast model of Parkinson's disease*
12:45 – 13:45	Lunch break

	Session 3 Chair: Dina Hofer, Katharina Huber
13:45 – 14:30	**Plenary Lecture: Carles Cantó** **(Nestlé Institute of Health Science, Lausanne, Switzerland)** *Mediation of adipose tissue lipolytic processes by mitofusin 2 (Mfn2) impacts on whole-body energy homeostasis*
14:30 – 14:45	**Oral Presentation 7: Emilia Strandback** *Rescuing the stability of a cancer associated variant of human NQO1 by small-molecular chaperones*
14:45 – 15:00	**Oral Presentation 8: Eveline Adam** *The cultivar-specific seed microbiome of the Styrian oil pumpkin and implications for breeding and seed treatment technologies*
15:00 – 16:00	**Guided Poster Session**, Coffee break
	Session 4 Chair: Torben Kühnast, Lisa Katharina Maresch
16:00 – 16:45	**Plenary Lecture: Meta Kuehn** **(Duke University, North Carolina, US)** *Diverse mechanisms lead to a diversity of outer membrane vesicles*
16:45 – 17:00	**Oral Presentation 9: Verena Kohler** *TraN: a novel repressor of an Enterococcus conjugative type IV secretion system*
17:00 – 17:15	**Oral Presentation 10: Aline Telzerow** *Application of two new (R)-selective amine transaminases in a one-pot cascade reaction for the synthesis of drug-precursors*
17:15 – 17:45	**Closing remarks & awards**
17:45	**Get together with food and drinks**

CONFIDENTIALITY

The abstracts and presentations of the DocDay symposia contain unpublished data. Please respect these data as confidential information for the purpose of scientific discussion at today's meeting. Communications of the abstracts or research results outside of the Doctoral School of Molecular Biosciences and Biotechnology and the Doctoral School of Molecular Biology and Biochemistry is inappropriate.

GUEST SPEAKERS

(Chronological order)

Dr. Alexandros Vegiopoulos

Junior Group Metabolism and Stem Cell Plasticity
German Cancer Research Center
Heidelberg, Germany

Biography

Dr. Alexandros Vegiopoulos is head of the junior group "*Metabolism and Stem Cell Plasticity"* at the German Cancer Research Center (DKFZ) in Heidelberg. He obtained his Master's degree in biology at the Albert-Ludwigs University Freiburg and awarded his Ph.D. in 2005 at the University of Oxford/Birmingham in immunology/hematopoiesis. Afterwards he moved on to Heidelberg for a postdoctoral fellowship at the DKFZ where he first became team supervisor of the division "*Molecular Metabolic Control"* in 2010 and later junior group leader.

Research Interest

The focus of his work is the understanding of stem cell regulation by changes of systemic metabolism. In particular, he concentrates on adipose tissue mesenchymal stem/stromal cells (ASCs), which are immature cells partly associated with vasculature. These cells contribute to tissue remodeling by adopting different fates including the formation of new adipocytes. These cells play a crucial role in nutrition-driven early adipose tissue growth, leading to childhood obesity and the programming of adult metabolic disease. Additionally, he also contributes to a great extent to the discovery on regulation of the oxidative/thermogenic adipocyte fate of ASCs, which get activated by transient inflammatory stress signaling and prolonged cold exposure.

Lorenzo Galluzzi, Ph.D.

Assistant Professor of Cell Biology in Radiation Oncology
Weill Cornell Medical College
New York, US

Associate Professor of *Université Paris Descartes*
Paris, France

Biography

Lorenzo Galluzzi received his Ph.D. in 2008 in oncological sciences from the University of Paris Sud/Paris XI (France), and now works as assistant professor of cell biology in radiation oncology at Weill Cornell Medical College (New York, NY, US). He is also associate professor at Université Paris Descartes (France) and director of the European Academy for Tumor Immunology (EATI). Besides being part of the editorial board of *Oncoimmunology, Cell Stress, Microbial Cell, and Cell Death and Disease,* Dr. Galluzzi operates as editor-in-chief for *OncoImmunology*, *Molecular and Cellular Oncology, International Review of Cell and Molecular Biology and Oncotarge - Autophagy and Cell Death.* Dr. Galluzzi has published more than 300 articles in peer-reviewed scientific journals, and he is the 6th and youngest of the 30 most-cited European cell biologists (relative to the period 2007–2013).

Research interest

Dr. Galluzzi is interested in several aspects of cell biology, encompassing (i) the role of autophagy in malignant transformation, tumor progression and response to therapy (ii) system biology of mitochondrial apoptosis linked to the resistance to cisplatin of cancer cells (iii) oncometabolism (iv) immunogenic cell death and (v) tumor immunology. Dr. Galluzzi provided major contributions to the discovery that adaptive responses to cellular stress, including autophagy, are key determinants for the recognition of dying cancer cells as immunogenic, and hence for the activation of clinically relevant tumor-targeting immune responses in the context of chemotherapy and radiation therapy.

Cales Cantó, Ph.D.

Junior Group Leader, Specialist in Metabolism and Diabetes
Nestlé Institute of Health Sciences (NIHS)
Lausanne, Switzerland

MER at the École Polytechnique Fédérale de Lausanne (EPFL)
Lausanne, Switzerland

Biography

Dr. Cantó received his Ph.D. in 2007 in biomedicine from the University of Barcelona (Barcelona, Spain). From 2007 to 2011, he worked as a post-doctoral scientist at École Polytechnique Fédérale de Lausanne (EPFL) (Lausanne, CH). His excellent work on energy sensing and transcriptional adaptation to energetic stresses was granted with the “Fondation Latsis Internationale”-prize (2010). Currently, he leads the “Metabolic Sensing and Adaptation” group at the Nestlé Institute of Health Sciences (Lausanne, CH) and works as senior scientist for teaching and research at EPFL (Lausanne, CH). Dr. Cantó holds several patents on compounds and methods for the treatment of diseases related to insulin resistance and mitochondrial dysfunction. Besides his function as topic editor for *Molecular Metabolism* (Elsevier), he is lead reviewer for journals such as *Cell Metabolism* or *Molecular Cell*. Dr. Cantó has published more than 50 articles, thereof 20 in high-impact journals.

Research interest

The mission of Dr. Cantó is to understand how regulatory enzyme activities can sense nutritional and energetic cues and how this information can be used to promote metabolic adaptations and grant metabolic flexibility. The key goals of his group are (i) to understand the acute actions of how enzymatic sensors transfer their information, (ii) to evaluate how mitochondrial modifications influence metabolic flexibility, (iii) to understand how metabolic flexibility can be modulated at the transcriptional level, (iv) and to find nutritional possibilities to improve metabolic flexibility. This is important to prevent or treat complex metabolic diseases, such as type 2 diabetes and obesity.

Margarethe (Meta) J. Kuehn, Ph.D. Duke University School of Medicine

Associate Professor of Biochemistry, Molecular Genetics and Microbiology
Duke University
Durham, North Carolina, USA

Biography

Dr. Meta Kuehn received her Ph.D. at the Washington University in 1993. From 1994 to 1997 she performed as Howard Hughes postdoctoral research fellow at the University of California. Afterwards she joined the Duke University as associate professor of biochemistry and in molecular genetics and microbiology. In 1999 she also became codirector for the Duke Summer Research Opportunities Program (SROP) for undergraduate underrepresented minorities, in 2016 director of graduate studies.

Research interest

In her lab Dr. Meta Kuehn focuses on genetic, biochemical and functional features of bacterial vesicle production. Using a genetic screen, she and her team have identified genes essential in the vesiculation process, specific proteins that are enriched in vesicles and critical molecules that govern the internalization of vesicles into host cells. Using biochemical analysis of purified vesicles, she has found that heat-labile enterotoxin, an important virulence factor of ETEC, is exported from the cells bound to the external surface of vesicles. Presented in this context, it is able to mediate the entry of the entire ETEC vesicle into human colorectal tissue culture cells. She has also discovered that the ability of vesicles to bind to specific cell types depends on their strain of origin: for example, *P. aeruginosa* vesicles produced by a strain that was cultured from the lungs of a patient with Cystic Fibrosis adhered better to lung than to gut epithelial cells. The vesicles stimulate epithelial cells and macrophages to elicit a cytokine response that is distinct from that of LPS (a major component of the vesicles) alone.

ORAL PRESENTATIONS

(Chronological order)

Metabolic stem cell responses and the plasticity of fat

Alexandros Vegiopoulos
DKFZ Junior Group Metabolism and Stem Cell Plasticity, Heidelberg, Germany

The quantity and quality of adipose tissue is a major determinant in the pathogenesis of the metabolic syndrome and the associated progression to diabetes and cardiovascular disease. The remarkable plasticity of adipose tissue depends to a great extent on mesenchymal stem/stromal cells (ASCs). The regulation of ASC function by nutrition, organismal metabolism and the microenvironment as well as their contribution to adaptation and pathogenesis are a major current question in the field.
In previous and ongoing work we have revealed how transient inflammatory stress signaling in the adipose tissue microenvironment mediates the activation of ASCs for thermogenic adipocyte differentiation, which contributes to tissue remodeling and protects from metabolic disease.
Our most recent findings have addressed the control of ASC proliferation by nutrition in the development of childhood obesity. Here we are dissecting how nutritional components trigger ASC responses through systemic and microenvironmental signals and how these early postnatal responses program the susceptibility to metabolic disease in adulthood.

Identification and characterization of cancer-associated cachexia inducing factors

Ursula Feiler[1], Martina Schweiger[1] and Rudolf Zechner[1]

[1]Institute of Molecular Biosciences, University Graz, Graz, Austria

Cancer-associated cachexia (CAC) is a devastating, multifactorial syndrome characterized by a metabolic shift towards an extreme catabolic state. About 50% of all cancer patients suffer from CAC. These patients show an excessive body weight loss, mainly due to loss of muscle and adipose tissue which often leads to premature death. Until now, the molecular mechanisms causing uncontrolled loss of body mass are only poorly understood.
The aim of this study is to identify novel factors secreted from the tumor and/or from the host which induce lipid hydrolysis and protein degradation. We use two different CAC models: (i) the colon epithelial carcinoma cells C26 in CD2/F1 mice and (ii) the chemically-induced MCA207 cells in C57Bl/6J mice. There exist two variants of C26 and MCA207 cells, one leading to cachexia and one that does not. The inoculation of both CAC inducing cells caused a massive decrease of adipose and muscle tissue associated with an inflammatory response.

Using conditioned medium from cachexia-inducing and non-inducing tumors, we observed a stimulating effect on adipocytes lipolysis from both variants. Plasma from cachectic mice increased the glycerol release from fat pads as compared to plasma from non-cachectic mice, indicating the presence of pro-lipolytic factors in the bloodstream of the mice. Muscle atrophy was considered to be the cause of increased protein degradation by the proteasomal pathway. We could show that C26- and CHX207- tumor bearing mice exhibited increased expression of the two E3-ligases Atrogin-1/MAFbx and MuRF1, responsible for ubiquitination of proteins. Behind the molecular alterations, the inflammatory response is considered to be the main driving force in CAC. We observed elevated plasma levels of the cytokine interleukin-6 (IL-6) in cachectic mice as compared to non-cachectic mice, with the highest expression in tumor tissue.

To gain more insights into the molecular mechanisms causing CAC, we want to determine the differences between cachexia-inducing and non-inducing tumors using transcriptomic and metabolomic analyses.

Acknowledgements: This work is funded by the Austrian Science Fund FWF(W901) DK Molecular Enzymology and the European Research Council ERC Advanced Grant 2013 (LIPOCHEX Nr. 340896)

UCP1 deficiency in cancer-associated cachexia

Pia Benedikt[1], *Martina Schweiger*[1], *Rudolf Zechner*[1]
[1]*Institute of Molecular Biosciences, University of Graz, Graz, Austria*

Cancer-associated cachexia (CAC) is a multifactorial syndrome characterized by a significant reduction in body weight, resulting predominantly from loss of adipose tissue and skeletal muscle mass. About half of all cancer patients suffer from cachexia, which impairs quality of life, limits cancer therapy and decreases overall survival rate. Cachectic patients may ingest less food, but they are also in a hypermetabolic state with increased energy expenditure resulting in a negative energy balance. Besides activation of thermogenesis in interscapular brown adipose tissue (iBAT) a phenotypic switch, termed white adipose tissue (WAT) browning, has been observed in cachectic mice. Importantly, this browning effect was also observed in WAT from CAC patients, which underlines the translational value. This phenomenon is associated with increased expression of uncoupling protein 1 (UCP1) in WAT depots, which uncouples mitochondrial substrate oxidation from ATP synthesis. During this uncoupling process energy is dissipated as heat, leading to increased lipid mobilization and energy consumption.

To investigate the role of UCP1 deficiency in CAC, the K5-SOS knockout mouse model was bred into a UCP1 null background. This mouse model expresses a dominant form of hSOS (SOS-F) under the control of keratinocyte promotor K5, which leads to the development of spontaneous skin tumors. The K5-SOS mouse model is from special interest, because it shows increased activation of iBAT and browning of WAT during the progression of cachexia.

Global knockout of UCP1 in K5-SOS mice does not prevent or ameliorate the loss of WAT and skeletal muscle. Interestingly, UCP1 deficiency leads to the induction of thermogenic programs in inguinal WAT, causing a brown phenotype with increased mitochondrial content and multilocular lipid droplets. Further analysis revealed that impaired thermogenic activity of iBAT in K5-SOS/UCP1 knockout mice initiates a compensatory mechanism leading to increased FGF21 expression and secretion. Recent publications showed that UCP1 deficient iBAT secretes FGF21 to induce thermogenic and lipolytic gene expression in iWAT.

Molecular mechanism under browning in cancer associated cachexia

Hao Xie[1], Ursula Feiler[1], Pia Benedikt[1], Martina Schweiger[1], Rudolf Zechner[1]
[1]Institute of Molecular Biosciences, University Graz, Graz, Austria

Cachexia, a wasting syndrome characterized by systemic inflammation, body weight loss, atrophy of white adipose tissue (WAT) and skeletal muscle, is a serious problem that interferes with response to treatment and affects quality of life. Cachexia is commonly observed in a majority of individuals with advanced cancer, AIDS, tuberculosis, congestive heart failure, chronic obstructive lung disease, and multiple sclerosis, contributing to a great percentage of morbidity and mortality from these diseases. However, little is known about the underlying mechanisms of cachexia and limited effective treatment is currently available. Previously, our group showed that a phenotypic switch from white adipocyte to brown adipocyte, a phenomenon termed WAT browning, takes place in the initial stages of different cancer associate cachexia (CAC) models. Notably, systemic inflammation and β-adrenergic stimulation were found to be responsible for WAT browning, promoting the induction of energy expenditure and wasting syndrome.

Catecholamines are important signaling molecules for WAT browning, as well as for the stimulation of lipolysis. Tyrosine hydroxylase (TH) is the rate limiting enzyme responsible for catecholamine synthesis. Catechol-O-methyltransferase (COMT) is one of the most important enzymes that degrade catecholamines. We found that TH protein expression is significantly upregulated, while COMT protein expression is decreased in the stromavascular fraction (SVF) of adipose tissues only in cachectic animals. This observation is in line with accumulated catecholamine levels in cachectic adipose tissues when compared to non-cachectic or control adipose tissues. In cultured macrophages, we saw TH expression induced by pro-inflammatory stimuli. Importantly, conditioned medium from cultured macrophages overexpressing TH induces thermogenic gene expression in cultured white adipocytes. Hence we suggest that in mice bearing a cachexia inducing tumor, inflammatory stimuli lead to the activation of macrophages or neurons in the SVF of adipose tissues that produce catecholamines which in turn stimulate lipolysis and initiate browning.

Inside-Out – A story of intracellular stress and organismal homeostasis

Lorenzo Galluzzi
Weill Cornell Medical College, New York , US

Cancer cells, as other cells, die in the context of failing responses to stress. Such a regulated form of cell death is important not only as it purges the organism of useless and/or potentially dangerous cells, but also as it communicates to the immune system a state of incipient danger via damage-associated molecular patterns (DAMPs). DAMP emission by dying cancer cells and detection by the immune system is fundamental for the establishment of a therapeutically relevant tumor-specific immune responses. Several strategies have been employed to boost the efficacy of cancer (immuno)therapy. One of the least investigated of these approaches does not aim at changing the number of cancer cells succumbing to therapy, but at altering how these cells are perceived by the immune system by targeting premortem stress responses.

The role of N-acetyltransferase 8-like (Nat8l) in energy homeostasis and autophagy

Huber K.[1], Panzitt K.[2], Trausinger G.[3], Duta-Mare M.[4], Stryeck S.[4], Kolb D.[5], Magnes C.[3], Madl T.[4], and Bogner-Strauss J.G[1].

[1]Institute of Biochemistry, Graz University of Technology, Austria.

[2]Division of Gastroenterology and Hepatology, Medical University of Graz, Austria.

[3]Joanneum Research GmbH, HEALTH, Austria.

[4]Institute of Molecular Biology and Biochemistry, Medical University of Graz, Austria.

[5]Core Facility Ultrastructure Analysis, Medical University of Graz, Austria.

Autophagy is a catabolic process involving the lysosomal degradation of cytoplasmic organelles and components. During starvation, cells rely on proficient autophagic responses to replenish energetic requirements for cellular survival.
N-acetyltransferase 8-like (Nat8l) catalyzes the synthesis of N-acetylaspartate (NAA) from L-aspartate and acetyl-CoA. NAA is suggested to be a major storage and transport form of acetyl-CoA and aspartate and therefore an important player regulating the availability of metabolites for energy homeostasis. Nat8l is highly expressed in adipose tissues and localized in the mitochondria of brown adipocytes. To date, the physiological function of Nat8l remains elusive. Thus, we aim to investigate the role of Nat8l in energy metabolism and autophagy.

Here we report that overexpression of Nat8l (Nat8l o/e) leads to reduced levels of cytosolic acetyl-CoA and its precursors acetate and branched-chain amino acids indicating a fasting state in these cells. Concomitantly, mTORC1, a factor highly regulated by the nutritional cellular state, is inhibited in Nat8l o/e iBACs. mTORC1 inactivation increases transcription factor EB translocation to the nucleus, which supports lysosomal biogenesis. Additionally, autophagic flux is increased to compensate energy deficiency during starvation. Moreover, acetate supplementation could rescue the increased autophagic phenotype in Nat8l o/e cells to control levels.
Our data suggest that deregulation of the Nat8l/ NAA pathway regulates acetyl-CoA and amino acid availability and thereby impacts autophagy.

Acetyl-CoA Carboxylase as key regulator of autophagy in the yeast Saccharomyces cerevisiae.

Angelina Gross[1], Sabrina Schroeder[1], Tobias Pendl[1], Andreas Zimmermann[1], Alexandra Harger[2], Birgit Michelitsch[1], Martina Wölflingseder[1], Christina Kast[1], Sandra Ortonobes Lara[1], Hannes Schönlechner[1], Kirsten Harmrolfs[3], Rolf Müller[3], and Tobias Eisenberg[1]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria

[2]Medical University of Graz, Department of Internal Medicine, Graz, Austria

[3]HIPS Helmholtz-Institute for Pharmaceutical Research Saarland, Saarland University, 66123 Saarbrücken, Germany

Autophagy, a cellular process to remove and recycle superfluous and damaged cellular material, has been linked to healthy ageing.
Previously we showed that excess nucleo-cytosolic Acetyl-CoA (AcCoA) functions as a rheostat, regulating autophagy during starvation and ageing. High AcCoA levels promote acetylation of histones and cytosolic proteins, leading to repression auf autophagy genes, further inhibiting the autophagic flux and impairing chronological life span.

In our current work we aim to unravel a potential link of lipid and AcCoA metabolism in the regulation of life span and autophagy. Therefore, we investigate the role of the Acetyl-CoA Carboxylase 1 (Acc1) in yeast. This enzyme catalyzes the first step in *de novo* lipid-biosynthesis by carboxylation of AcCoA to produce malonyl-CoA, which is further used for chain elongation of fatty acids. We modulate the activity of Acc1 through various genetic and pharmacological means and assess metabolic changes and their implication in the regulation of ageing and autophagy.

We show that Acc1 activity plays a crucial role in both processes. An increased flux of AcCoA towards lipid-synthesis is sufficient to promote autophagy, whereas inhibition of Acc1 strongly decreases life span and inhibits autophagy. We further aim at investigating the complex mechanisms, by which Acc1 and fatty acid metabolism are orchestrated to efficiently maintain healthy ageing and control the autophagic flux.

This work helps us to understand the complex interplay of the main energy generating circuitries in the regulation of autophagy. It will not only be helpful for understanding the ageing processes but also related diseases with a high need in energy supply such as cancer.

LRRK2 impairs autophagy and endocytic pathways in a yeast model of parkinson's disease

Andreas Aufschnaiter[1], Verena Kohler[1], Elisabeth Wachter[1], Marie Zechner[1], Walter Keller[1] and Sabrina Büttner[1,2]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria

[2]Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

Genetic variations of the leucine-rich repeat kinase 2 (LRRK2) present the most prevalent cause of familial Parkinson's disease (PD). Recent studies link an impairment of autophagy to the kinase and/or GTPase domain of LRRK2, which also harbor the most prevalent pathogenic mutations. Thus, we evaluated the effects of the catalytic core of LRRK2, consisting of a Ras-like GTPase (ROC), a C-terminal of ROC (COR) and a kinase domain, together named LRRK2-GCK, during ageing.

In the present study, we created a PD-yeast model heterologously expressing human LRRK2-GCK and studied oxidative stress, viability, autophagy and vesicular trafficking via flow cytometry, fluorescence microscopy, immunoblotting and other biochemical assays. Already within the first day of chronological ageing, we observed oligomeric forms of LRRK2-GCK. With progressing cellular age, this protein caused a severe impairment of autophagy and distinct endocytic pathways, subsequently resulting in increased levels of reactive oxygen species and finally cell death. Interestingly, the level of cytotoxicity varied between the different PD-associated mutant forms of LRRK2.

In this work, we provide evidence for a critical role of the GCK-fragment in the dysregulation of autophagy and distinct endocytic pathways ultimately resulting in cell death. To shed light on the molecular details of these processes, we aim for purification of this protein, followed by biophysical and structural characterization to connect these attributes to its toxic effects.

Mediation of adipose tissue lipolytic processes by mitofusin 2 (Mfn2) impacts on whole-body energy homeostasis

Carles Cantó

Nestlé Institute of Health Sciences, Lausanne, Switzerland

Mitochondrial fusion and fission events, collectively known as mitochondrial dynamics, act as quality control mechanisms to ensure mitochondrial function and fine-tune cellular bioenergetics. Defective mitofusin 2 (Mfn2) expression and enhanced mitochondrial fission in skeletal muscle are hallmarks of insulin resistant states. Interestingly, Mfn2 is highly expressed in brown adipose tissue (BAT), yet its role remains unexplored. Using adipose-specific Mfn2 knockout (Mfn2-adKO) mice we demonstrate that Mfn2 deficiency in BAT leads to a profound BAT dysfunction, associated with impaired respiratory capacity and a blunted response to adrenergic stimuli. Importantly, Mfn2 directly interacts with perilipin, facilitating the interaction between the mitochondria and the lipid droplet in response to adrenergic stimulation. Surprisingly, Mfn2-adKO mice were protected from high-fat diet-induced insulin resistance and hepatic steatosis. Altogether, these results demonstrate that Mfn2 is a mediator of mitochondria to lipid droplet interactions, influencing lipolytic processes and whole-body energy homeostasis.

Rescuing the stability of a cancer associated variant of human NQO1 by small-molecular chaperones

Emilia Strandback[1], Wolf-Dieter Lienhart[1], Jakob Pletz[2], Andreas Winkler[1], Klaus Zangger[3], Karl Gruber[4], Rolf Breinbauer[2], Peter Macheroux[1]

[1]Institute of Biochemistry, Graz University of Technolog, Graz, Austria

[2]Institute of Organic Chemistry, Graz University of Technology, Graz, Austria

[3]Institute of Chemistry, University of Graz, Graz, Austria

[4]Institute of Molecular Biosciences, University of Graz, Graz, Austria

NAD(P)H:quinone oxidoreductase 1 (NQO1; EC 1.6.99.2) is a human FAD dependent enzyme catalyzing the two-electron reduction of quinones to hydroquinones. NQO1, for instance, plays an important role in the antioxidant defense system where it lowers the quinone levels and thereby prevents the formation of reactive oxygen species (ROS). Furthermore, it binds to the 20*S* proteasome, thus stabilizing several tumor suppressors like $p33^{ING1b}$, p53 and p73. The level of NQO1 is also increased in several tumors, therefore it is an important target for anti-cancer prodrugs.

A naturally occurring single nucleotide polymorphism (*NQO1*2*) in the *NQO1* gene, results in the replacement of proline 187 to serine (P187S) in the protein sequence. The distribution of the homozygous *NQO1*2* among the population varies between 2 and 20% depending on the ethnical background. The reduced activity and stability of the NQO1 P187S variant leads to an increased toxicity of benzene, higher risk for several types of cancers and poor survival rate after anthracycline-based chemotherapy.

A previous study gave insights into the structural characteristics of the NQO1 P187S variant and concluded that the single amino acid exchange destabilizes interactions between the core and C-terminal domain of the variant protein in solution. The ongoing studies involve further investigations of the behavior of the NQO1 P187S variant as well as the possibilities to find a way to restore the activity and stability of the NQO1 P187S variant by small molecules. The first results show that the investigated compound binds to and stabilizes the NQO1 P187S variant.

The cultivar-specific seed microbiome of the Styrian oil pumpkin and implications for breeding and seed treatment technologies

Eveline Adam[1,2], Maria Bernhart[1,2], Henry Müller[1], Johanna Winkler[1], Gabriele Berg[1]
[1]Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria
[2]Saatzucht Gleisdorf GmbH, Gleisdorf, Austria

Collectively known as the plant microbiome, plant-associated microbes can help plants to fend off diseases, stimulate growth, occupy space that would otherwise be taken up by pathogens and promote stress resistance. Additionally they influence crop yield and quality. The specific arrangement of plant-associated microbiomes is supposed to be a consequence of breeding activities and genotype selection.
To support a concept involving beneficial plant-microbe interactions in breeding activities of Styrian oil pumpkin, the microbiomes of seeds and rhizospheres of 14 genotypes as well as the bulk soil were analyzed using a 16S rRNA gene amplicon sequencing approach.

In general, the diversity of the observed operational taxonomic units (OTUs) in rhizosphere samples was significantly higher than in seed samples and a strong genotype specificity was detected for the seed-associated microbial communities. The seed core microbiome of the cultivars analyzed in this study was dominated by high abundances of seven *Enterobacteriaceae*, one *Pseudomonadaceae*, one *Lactococcus* and one *Exiguobacterium* OTU. In two lines the microbiome was comprised, to a large extent, of the genus *Erwinia* as well as the important pathogen *Pectobacterium carotovorum* (syn. *Erwinia carotovora*), the causal agent of soft rot of fruits. Potential plant-beneficial bacteria like *Lysobacter*, *Paenibacillus* and *Lactococcus* contributed to the microbial communities as well. Three cultivars showed a significant higher microbial alpha-diversity in their seeds as well as a higher Heip evenness index than the other eleven genotypes investigated.

The results of the seed analysis are of particular interest for the seed production industry, as the Styrian oil pumpkin is highly susceptible to various fungal and bacterial pathogens during germination making chemical or complex seed treatments inevitable. It remains to be investigated to which extent naturally occurring seed-borne bacteria influence germination and plant development. Those results could direct the design of tailored biological seed treatments or influence seed disinfection strategies that might replace fungicide treatments in future. A possible implication for breeding programs could be the selection of genotypes enriching less enterobacteriaceal pathogens and/or expressing a higher microbial diversity in their seeds.

Diverse mechanisms lead to a diversity of outer membrane vesicles

Meta Kuehn
Duke University, North Carolina, US

Outer membrane vesicles (OMVs) are heterogenous spherical proteoliposomes constitutively produced by all Gram-negative bacteria. OMVs bud and release from the outer membrane (OM) of cells, encapsulating periplasmic material. OMVs are approximately 50 to 300 nm in diameter and contain enriched envelope protein and lipid constituents. OMVs are now well-known to perform a diversity of functional roles, and their role in virulence factor dissemination are particularly well-characterized. The particular inclusion of lipid and protein cargo into OMVs impart a variety of functions. However, the mechanics that promote OM bulging and OM fission remain elusive. Using genetic and biochemical assays, the mechanistic details of OM biogenesis and relationships between LPS structure and OMV-cargo inclusion rates are now shedding light on how the OM is organized, how OM fluidity changes, how the OM is remodeled based on environmental changes, and how these impact both the amount and content of the budding vesicles. Peptidoglycan structure, turnover, and covalent crosslinking between the PG and the OM play a role in OMV biogenesis and regulation. Also, specific LPS binding by extracellular protein can impact OMV production levels. In other cases, OMV production is driven by an increase in periplasmic pressure. In sum, various biophysical parameters and biochemical pathways influence OMV production. As we uncover more mechanistic details about OMV production and identify key factors involved in this process, we better understand the universal process of membrane vesiculation, and, specifically, the impact of OMV production on bacterial pathogenesis.

TraN: a novel repressor of an *Enterococcus* conjugative type IV secretion system

Verena Kohler[1], Andreas Aufschnaiter[1], Sabrina Büttner[1,2], Ines Probst[3], Elisabeth Grohmann[3,4] and Walter Keller[1,]*

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria.

[2]Department of Molecular Biosciences, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.

[3]Division of Infectious Diseases, Department of Internal Medicine, University Medical Center Freiburg, Freiburg, Germany.

[4]Faculty of Life Sciences and Technology, Beuth University of Applied Sciences, Berlin, Germany.

Spreading of antibiotic resistances in bacteria is an enormous issue nowadays, causing tremendous problems in healthcare, and Gram-positive (G+) pathogens are among those most responsible for nosocomial infections. However, scarce information about systems responsible for dissemination of drug-resistances in G+ is available. Based on its prevalence in numerous bacterial pathogens, we are studying the conjugative model plasmid pIP501, coding for 15 transfer-genes (*tra*-genes) of a simplified type IV secretion system (T4SS).
With the final goal of gaining novel information regarding conjugative transfer mechanism in G+ hosts, our group focuses on biochemical and structural characterization of the pIP501 T4SS.

In this work, we establish TraN, a small cytoplasmic Tra-protein, as repressor of the whole conjugation machinery. Promoter activity assays verified the proposed binding site of TraN on pIP501 plasmid. In-frame deletion of *traN* led to increased transfer frequencies observed in *E. faecalis* mating experiments. When comparing mRNA as well as protein levels of wild type and knock-out strain, we observed a significant increase in *tra*-gene expression levels upon *traN* deletion. This finding was further evidenced with immunofluorescence microscopy approaches.

These data suggest that TraN plays a major role in regulation of *tra*-gene expression as well as in conjugative plasmid transport and we can hereby state that this protein is a novel repressor of the pIP501 T4SS.

Application of two new (*R*)-selective amine transaminases in a one-pot cascade reaction for the synthesis of drug-precursors

A. Telzerow[1], M. Håkansson[2], M. Schürmann[3], H. Schwab[1], K. Steiner[4]

[1]Institute of Molecular Biotechnology, TU Graz, Graz, Austria

[2]SARomics Biostructures AB, Lund, Sweden

[3]DSM, Innovative Synthesis, Geleen, The Netherlands

[4]acib GmbH, Graz, Austria

(*R*)-selective amine transaminases are promising biocatalysts for the production of chiral amines. In general, aminotransferases are pyridoxal-5′-phosphate (PLP)-dependent enzymes, which reversibly catalyze the transfer of an amino group from an amino donor to a ketone or aldehyde resulting in the formation of chiral amines. These amines often serve as drug-precursors.

The aim of this work is the production of a drug-precursor for the treatment of osteoporosis in a one-pot cascade reaction. The cascade consists of a chemo- and a biocatalytic step. The biocatalytic step is a bio-amination reaction for which new (*R*)-selective amine transaminases are needed. Applying database searches five new transaminases were identified. Characterizations showed that the transaminases *Pac* and *Shi* catalyze the target reaction in the cascade. To further improve their activity enzyme engineering methods are used. For a rational approach the crystal structure of *Shi* has been determined to a resolution of 2.1 Å.

POSTER PRESENTATIONS

(Alphabetical order)

ATGL deficiency leads to lung cancer in mice

Bina, I.[1], Al-Zoughbi, W.[1], Schauer, S.[1], Stadelmeyer, E.[1], Nicholson Puthen Veedu, S.[1], Lana, A.[1], Marsh, L.[3], Schlederer, M.[4], Schweiger, M.[2], Casanova, E.[4], Kenner, L.[4], Zechner, R.[2], Hoefler, G.[1] and Vesely, P.W.[1,2]

[1]*Medical University of Graz, Institute of Pathology, Graz, Austria*

[2]*University of Graz, Institute of Molecular Biosciences, Graz, Austria*

[3]*Ludwig Boltzmann Institute Lung Vascular Research, Graz, Austria*

[4]*Ludwig Boltzmann Institute Cancer Research, Vienna, Austria*

Metabolic reprogramming represents a hallmark of malignant cell transformation. Rapidly proliferating cancer cells exhibit high rates of *de novo* lipogenesis. The role of the lipolytic cascade and its rate limiting enzyme adipocyte triglyceride lipase (ATGL), in cancer development and progression however, has not been sufficiently investigated so far.

With this study we want to shed light upon the role of ATGL, the rate limiting enzyme of lipolysis, in cancer development and progression.
To decipher a possible function of ATGL in *de novo* tumor formation we decided to age ATGL deficient mice (*Atgl*KO-cm*Atgl*TG). Unexpectedly, ATGL absence provoked development of multi-focal bronchial epithelial neoplastic lesions as well as invasive adenocarcinoma. This phenotype was accompanied by impaired lung function but also massive lipid accumulation in bronchial epithelial club cells (formerly clara), as identified by Oil Red-O staining, CCSP- and PLIN-1- immunohistochemistry. Additionally, the PPAR-α target gene *Cpt1α* was decreased in *Atgl*KO-cm*Atgl*TG lungs, indicating a mitochondrial energy usage impairment. Consequently, we observed decreased CCSP protein abundance in *Atgl*KO-cm*Atgl*TG lungs, which could trigger lung inflammatory processes. Therefore, we explored the status of the inflammatory IL-6-STAT3 signaling axis, which is very often deregulated in human lung cancer and serves as a repair mechanism in bronchial epithelial cells. Indeed, we observed increased IL-6 levels and phosphorylation of STAT-3 (Y705) in *Atgl*KO-cm*Atgl*TG lungs, especially in the bronchiolar epithelium, which gives rise to the neoplasia.

Therefore, we propose that energetic imbalance and subsequent impaired club cell function could eventually lead to hyperactive IL-6 signaling and regeneration related lung cancer development in animals lacking ATGL.

Bacterial bioluminescence: the *in vitro* formation of myrFMN

Eveline Brodl, Chaitanya R. Tabib and Peter Macheroux
Institute of Biochemistry, Graz University of Technology, Graz, Austria

Bioluminescence is the enzymatic production of light by living organisms. The enzyme luciferase is a heterodimeric protein consisting of a 40 kDa α-subunit and a 37 kDa β-subunit (LuxAB). The catalyzed reaction involves monooxygenation of a long-chain aliphatic aldehyde to the corresponding acid employing FMN as redox cofactor. The free energy released during this oxidation causes an excited state FMN-4a-hydroxide, which serves as the light emitting molecule luciferin. All proteins involved in bacterial bioluminescence are encoded by genes of the *lux*-operon. Specific strains in *Photobacteria* carry an extra gene termed *luxF*. LuxF is a homodimeric protein which can bind four flavin derivatives. These molecules exhibit an unusual carbon-carbon bond where the C-6 of the isoalloxazine ring is linked to the C-3 of myristic acid resulting in 6-(3'-(*R*)-myristyl)-FMN (myrFMN). It was postulated that myrFMN is a potential inhibitor of LuxAB.

The discovery of myrFMN raised the question of its formation. In this study myrFMN was examined *in vivo* and *in vitro*. To investigate the mechanism of bacterial bioluminescence, *Photobacterium leiognathi*, *Vibrio harveyi* and *Aliivibrio fisheri* were chosen as model systems to gain more information about the formation of this unusual FMN derivative.
A method for the isolation of myrFMN from *P. leiognathi* S1 was developed. *In vivo* analysis of different photobacterial strains revealed that myrFMN formation is independent of LuxF occurrence. The replication of the bioluminescent reaction *in vitro* was achieved by applying enzyme cascades with cofactor recycling systems. In multiple turnover reactions, exhibiting bioluminescence for more than 48 hours, the formation of myrFMN was confirmed via HPLC analysis.

The first time, it was possible to produce myrFMN *in vitro* and to confirm its formation by HPLC-MS analysis. The generation of myrFMN by luciferase supports the hypothesis of a radical mechanism.

Long-range allosteric regulation of GTP turnover in red-light modulated diguanylyl cyclases

G. Gourinchas, S. Etzl, and A. Winkler
Institute of Biochemistry, Graz University of Technology, Graz, Austria

Many organisms have the ability to respond to changes in environmental light conditions. Photoperception is allowed by a collection of modular photoreceptors that couple light sensing to allosteric regulating of signaling cascades critical for cell survival. Recently, photosensors coupled to enzymatic effectors have attracted special attention due to their potential for optogenetic applications. Among them, red-light activated phytochromes are promising due to deep-tissue penetration and low phototoxicity of red-light. However, the limited understanding of molecular mechanisms of light-signal transduction limits the rational design of innovative sensor-effector couples.

To improve our understanding of sensor-effector coupling we focus on naturally occurring red-light activated diguanylyl cyclases (PadC) that feature a canonical bacteriophytochrome fused to a GGDEF domain featuring diguanylyl cyclase activity involved in the production of the bacterial second messenger cyclic-dimeric-GMP. The phytochrome photointerconverts between a Pr and a Pfr state triggered by isomerization of its biliverdin chromophore upon red-light illumination. How this eventually alters the activity of downstream effector domains is still poorly understood.

We obtained the first crystal structure of a full-length phytochrome-effector couple revealing structural details of the sensor-effector connection, and substantiating the involvement of previously proposed important structural elements of phytochrome signaling. Moreover, using a combination of biochemical and integrative structural methods (X-ray, solution-scattering and HDX-MS) we highlight the importance of the sensor-effector linker composition for tuning the conformational dynamics required for signal integration and transduction.

Microbial investigation of milk product quality in different operating levels

Sandra Holzer[1] , Michael Murkovic[2]
[1]*Biomerx Vermögensverwaltung, Pasching, Austria*
[2]*Institute of Biochemistry, University of Technology, Graz, Austria.*

In this comprehensive study milk products, e.g. whey protein concentrate (WPC), unskimmed milk or low fat milk, were investigated during its whole production process. For this purpose samples were taken at specific production steps and microbial contamination was recorded. Also raw-material quality was proved, considering climate and delivery time.

The target is getting a better product quality, eliminate hygenic factors that may influence the quality of production and create a specific bacteria profile of the whole production-process.

Classical methods of microbiology were used for investigation, e.g. inoculation plate methods for bioburden, enrichment methods in combination with plate methods for *Cronobacter Sakazakii* – detection. In addition to classical methods, API and Riboflow were used to verify *Cronobacter*-suspect microbes on chromogenic agar plates.

In general raw product quality is much lower than final product- quality, in paticular WPC raw-products. In this case, a high percentage was tested *Cronobacter* positive. Also bioburden, *Enterobacteria* and *Coliforms* were present in a high number in all samples. After two or even three heating steps, the number of not-heat-resistant microbes decreased.Also *Cronobacter spp.* contaminated just raw product samples. Otherwise in the final products the number of thermophilic bacteria and thermophilic spores rose the same way as the non-thermophilic microbes decreased. The main target in this study was, to find out the specific bacteria-profile of each production-step and to use this knowledge to control product quality.

Regulation of Steryl Ester Metabolism in the Yeast S*accharomyces cerevisiae*

Korber M, Klein I, Ploier B, Schmidt C, Koch B, Athenstaedt K and Daum G
Institute of Biochemistry, Graz University of Technology, Graz, Austria

In the yeast, the two major non-polar lipids are triacylglycerols and steryl esters. They are formed by enzymes localized to the endoplasmic reticulum and stored in lipid droplets. Synthesis of triacylglycerols occurs by catalysis of the two acyltransferases Dga1p and Lro1p; and steryl esters are formed by the two steryl ester synthases Are1p and Are2p. Both types of non-polar lipids, triacylglycerol and steryl esters, can be mobilized from lipid droplets upon requirement. The triacylglycerol lipases Tgl3p, Tgl4p and Tglp5p mobilize triacylglycerols, and the steryl ester hydrolases Yeh1p, Yeh2p and Tgl1p cleave steryl esters. All three triacylglycerol lipases as well as Yeh1p and Tgl1p are localized to the surface of lipid droplets, whereas Yeh2p is rather found at the cell periphery.

The aim of recent studies was to shed some light on the regulation of steryl ester metabolism. In particular, we addressed the question how compromised degradation of steryl esters affected the synthesis of these lipids. For this purpose, a triple mutant deleted of *YEH1, YEH2* and *TGL1* was used, and the effect of these deletions on the steryl ester forming enzymes Are1p and Are2p was tested. Interestingly, a block in steryl ester hydrolysis does neither affect gene expression nor protein levels but the activity of steryl ester synthesizing enzymes. Hence, the *Δtgl1Δyeh1Δyeh2* triple mutation causes feedback inhibition to steryl ester formation. Another regulatory aspect on steryl ester metabolism investigated was the fate of the three steryl ester hydrolases in the absence or presence of lipid droplets. These findings are discussed as part of the overall network of non-polar lipid synthesis, storage and mobilization.

Supported by FWF projects 27346 and DK Molecular Enzymology W901-305 to G.D.

Investigating the active site of an oxidase ring closure enzyme from *Aspergillus fumigatus*

Majd Lahham[1], Bastian Daniel[1], Michael Fuchs[2], Tea Pavkov-Keller[3,4], Wolfgang Kroutil[2], Karl Gruber[3], and Peter Macheroux[1]

[1]*Institute of Biochemistry, Graz University of Technology, Graz, Austria*

[2]*Institute of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Graz, Austria*

[3]*Institute of Molecular Biosciences, University of Graz, Graz, Austria*

[4]*Austrian Centre of Industrial Biotechnology, Graz, Austria*

Flavoproteins constitute a versatile group of enzymes that employ the FAD or FMN co-factor and play major roles in the catalysis of different redox reactions. These cofactors are derived from riboflavin (vitamin B2) and comprise a redox-active isoalloxazine ring. Flavoproteins are broadly spread in all domains of life including fungi where they are mostly found in the saprotrophs. An oxidase – named FsqB - isolated from *Aspergillus fumigatus* was found in the biosynthetic pathway of the isoquinoline alkaloid fumisoquin. This enzyme has a homologous function to the berberine bridge enzyme (BBE) isolated from *Eschscholzia californica,* which has a bi-covalently linked FAD cofactor. The suggested step-wise mechanism of this enzyme is similar to BBE, in that a hydride is abstracted from the substrate to reduce the FAD. Then, a proton is abstracted from the phenol moiety enabling the nucleophilic attack on the iminum intermediate leading to the formation of the cyclic product. On the other hand, the same mechanism was suggested in a concerted manner. FsqB is similar to sarcosine oxidase (accession number EAL85695), a member of the D-Amino acid oxidase family, that is well known to operate by a hydride transfer mechanism from methyl group of the substrate to the FAD. However, a comprehensive understanding of FsqB oxidation mechanism still needs to be clarified. Studying the active site of the FsqB will provide more insight into the reaction mechanism. This study will be strengthened by docking experiments to predict how non-natural substrates bind to the active site and are oxidized by the FAD cofactor.

^{13}C labeling of lipids- tracing the flux of fatty acids in the yeast *S.cerevisiae*

D. Liebelt, G.N. Rechberger, S.D. Kohlwein

Institute of Molecular Biosciences, BioTechMed-Graz, University of Graz, Graz, Austria

The composition of cellular lipids is enormously complex; understanding how cells sense and adjust their lipid profiles during growth and differentiation, and how this translates to fatty acids being channeled towards either growth or storage pathways is of fundamental interest. In addition, deregulated cellular lipid homeostasis is associated with ailments like obesity, cardiovascular diseases and cancer, afflictions that have seen a dramatic rise during the past decades and require a better understanding of the underlying molecular mechanisms.

This project aims to establish an analytical routine allowing dynamic tracking of *de novo* fatty acid synthesis and lipid species fluxes, and to subsequently employ it to study lipidome kinetics in the model system, *S. cerevisiae*. To this end a combination of ^{13}C glucose labeling and mass spectrometric analysis of ensuing specific mass offsets is being developed.

Comprehensive labeling of neutral and phospholipids with ^{13}C glucose in a CEN.PK113-7D wild type strain is achieved in an overnight pre-culture. After shifting cells from ^{13}C glucose to ^{12}C glucose, exchange of labeled lipid species for unlabeled ones is observed. Appearance of intermediate species that harbor a combination of labeled and newly synthesized unlabeled acyl chains follows a dynamic rise and fall within 5h after shifting as does incorporation of newly synthesized headgroups and glycerol backbones. With this approach we are able to show that remodeling kinetics differ significantly between lipid classes, most dramatically between phosphatidylcholine (PC) and phosphatidylinositol (PI). Newly synthesized PI obtains its fatty acids from lipid turnover rather than *de novo* synthesis. In contrast, acyl chains in PC are remodeled by exchange with newly synthesized fatty acids. These data clearly demonstrate that *de novo* phospholipid synthesis does not follow a linear pathway via the intermediate phosphatidic acid.

A comparison of wild type (WT) and acyltransferase (AT) knock out strains shows differential remodeling kinetics which are currently further explored. These data help to elucidate the individual contribution of these AT enzymes to establishing specific lipid profiles and channeling of fatty acids into either membrane or storage lipids.

Boosting expression in a tailor-made way by new promoters and terminators for *S. cerevisiae*

Julia Pitzer[1,2], Raphaela Wimmer[1], Anton Glieder[1]

[1]*Institute of Molecular Biotechnology, Graz University of Technology, Graz, Austria*

[2]*Austrian Centre of Industrial Biotechnology (ACIB GmbH), Graz, Austria*

Efficient and well-regulated promoters and terminators are essential for the successful production of recombinant proteins and especially important for the implementation of enzymatic pathways e.g. for the biosynthesis of chemical building blocks. Highly effective transcriptional control is required for the fine-tuning of gene expression in heterologous hosts. In this study, new regulatory DNA elements were designed for *Saccharomyces cerevisiae* to expand the toolbox of available parts for transcriptional regulation.

Mono- as well as bidirectional promoters, which can be applied for divergent transcription of two genes, were investigated with different carbon sources. Sequence diversification of a bidirectional histone promoter based on a random-mutation walking library allowed the generation of a promoter library with a broad activity range. Thereby, variants with 200-300% increased promoter activity were obtained. Knowledge about neutral regions, without expression regulating effects, was utilized for the introduction of GAL4 binding site to equip the originally constitutive histone promoter with an additional galactose inducible element. In this way, a promoter variant with similar activity on glucose and strongly increased activity on galactose was created. Additionally, selected heterologous terminators were tested in *S. cerevisiae* and expression enhancing effects compared to standard native terminator sequences were discovered.

Acknowledgement

The research leading to these results has received funding from the Innovative Medicines Initiative Joint Undertaking under *grant agreement* n°115360, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. www.imi.europa.eu

Tracing metabolic evolution: From normal to cancerous

S. Nicholson Puthen Veedu[1], C. Madreiter-Sokolowski[3], D. Hofer[4], I. Bina[1], E. Stadelmeyer[1], S. Schauer[1], R. Zechner[2], J. Bogner-Strauss[4], W. Graier[3], G. Hoefler[1], and P. W. Vesely[1, 2]

[1] *Institute of Pathology, Medical University of Graz, Graz, Austria*

[2] *Institute of Molecular Biosciences, University of Graz, Graz, Austria*

[3] *Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria,*

[4] *Institute of Biochemistry, University of Technology, Graz, Austria.*

Carcinogenesis is a sequential process where cells acquire alterations on genetic, epigenetic, and metabolic levels. Using a murine embryonic fibroblast model, we break down this complex process into stages of cellular senescence, replicative immortality, and transformation. This enables us to trace the evolution of cellular metabolic processes of each phase and transition during carcinogenesis.

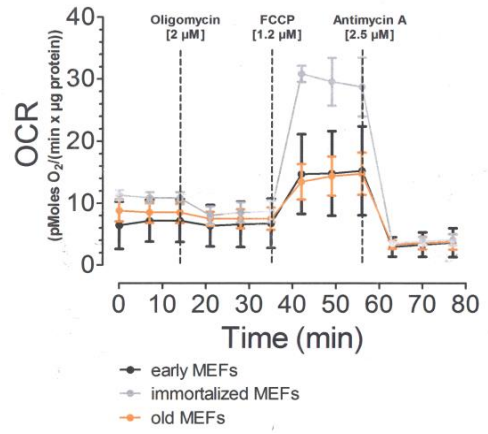


Figure 1. Oxygen consumption rate (OCR) of MEFs at different stages of carcinogenesis

We analyzed MEFs at progressive stages of carcinogenesis. “Early” MEFs (freshly isolated), “old” MEFs (cells in senescence) and “immortalized” MEFs. Immortalized MEFs showed a proliferative advantage over the early and senescent MEFs. On molecular level this is triggered by genetic switches including inactivation of p53 and $p16^{INK4A}$. On metabolic level, immortalized MEFs show increased maximal respiratory capacity (after FCCP injection) as measured by oxygen consumption rate by seahorse *(figure 1)* but only slightly enhanced lactate production and unchanged glucose uptake compared to MEFs in the other stages.

We propose that in this model of tumorigenesis, cells with extra mitochondrial capacity are selectively favored since this capacity is able to provide energy under conditions of metabolic challenge or stress, thereby enhancing cell survival and function. Detailed studies on the energy conversion pathways from bulks and single cells are currently performed and will shed light on the evolution of energy metabolism during early stages of carcinogenesis.

Studying the role of the only berberine bridge enzyme-like protein in *Physcomitrella patens*

Marina Toplak, Jelena Ulićević, Bastian Daniel, Silvia Wallner and Peter Macheroux
Institute of Biochemistry, Graz University of Technology, Graz, Austria

Berberine bridge enzyme- (BBE-) like proteins (pfam 08031) were shown to catalyze reactions in various metabolic pathways that are found in fungi, bacteria and plants. While bacteria and fungi possess just one or two homologues of a *bbe*-like gene, plants were found to accumulate a significant amount of homologues varying from one up to 57.
A plant carrying just one *bbe*-like gene is the model organism *Physcomitrella patens*, which is particularly interesting as this moss is the lowest developed plant harboring a gene for a BBE-like protein (*Pp*BBE) – due to that fact this gene is considered a potential common ancestor of the whole multigene family.

To broaden our understanding of the BBE-like protein family we initiated the characterization of the heterologously expressed enzyme: very important was the identification of *Pp*BBE being a cellobiose oxidase that catalyzes the conversion of cellobiose to the corresponding lactone.
For this reaction a pH optimum of pH 5.5-6 and a temperature optimum of 35°C were determined. In addition, temperature stability of the protein was analyzed, revealing a melting temperature of about 60°C.

Hence, it can be concluded that *Pp*BBE is a temperature stable disaccharide oxidase that catalyzes the oxidation of cellobiose to the corresponding lactone. Since cellobiose is an important degradation product of cellulose, which is one of the major components of the plant cell wall that is known to be of acidic pH, it is likely that also *Pp*BBE is found in the cell wall of the moss. This might explain, why the pH-optimum of *Pp*BBE is at around pH 6, which is lower than for most other BBE-like proteins studied so far.
To test this hypothesis characterization of a *Pp*BBE knock-out strain is currently going on.

NOTES

NOTES

ACKNOWLEDGEMENTS

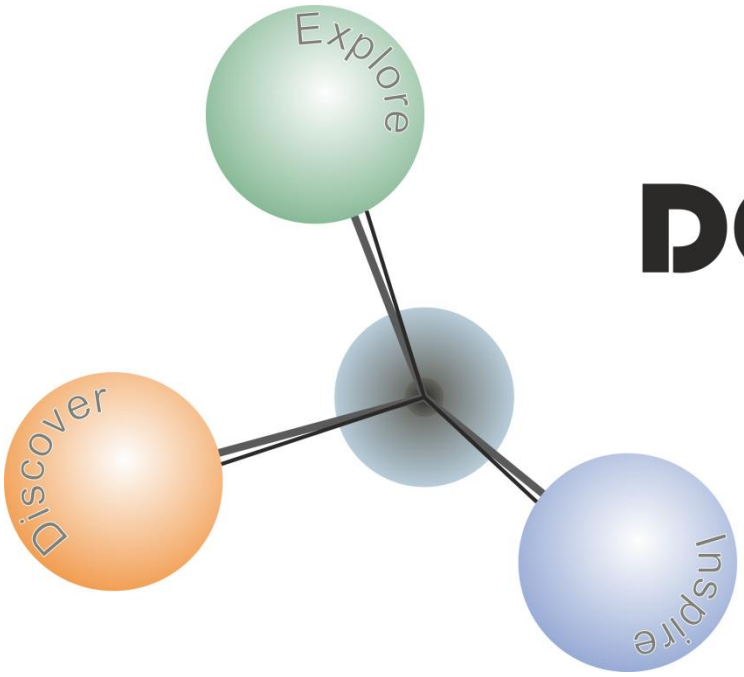
This DocDay is supported by

Doctoral School of Molecular Biosciences and Biotechnology

Doctoral School of Molecular Biology and Biochemistry

DK Molecular Enzymology

DK Metabolic and Cardiovascular Disease



DOC DAY
DD 17

We thank Felix Tobola for designing the DocDay logo