Eukaryotic Lipids;

Treasure of Regulatory

Information

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Poster abstracts

Poster abstracts (alphabetic)

The numbering refer to the numbers of the poster boards.
The presenter's name is underlined and only her/his lab is mentioned.
[#] *Selected for oral presentation.*

1. **Amara,** Sawsan
2. **Angelini,** Roberto
3. **Ayvazyan,** Naira
4. **Baksa,** Attila
5. [#]**Balreira,** Andrea
6. **Baranowski,** Marcin
7. **Barriga,** Hanna
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9. **Bilgin,** Mesut
10. [#]**Bohdanowicz,** Michal
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12. **Bozkurt,** Ozlem
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14. **Cano,** Ainara
15. **Cerrada de Dueñas,** Alejandro
16. **Chiritoiu,** Gabriela
17. **Delage,** Elise
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32. **Lissina,** Elena
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48. **Telenius,** Jelena
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50. **Weis,** Nicole
51. **Wilmes,** Claudia
52. **Wu,** Wen-Guey
53. **Zhao,** Hongxia

No poster

Heilmeyer, prof.dr. L., emeritus

1. Lipolysis of Natural Long Chain and Synthetic Medium Chain Galactolipids by Pancreatic Lipase-Related Protein 2

Sawsan Amara, Nathalie Barouh, Jérôme Lecomte, Dominique Lafont, Brice Fiorentino, Paul Boullanger, Sylvie Robert, Pierre Villeneuve, Alain De Caro, Frédéric Carrière

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Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are the most abundant lipids in nature, mainly as important components of plant leaves and chloroplast membranes. Pancreatic lipase related protein 2 (PLRP2) was previously found to express galactolipase activity, and it is assumed to be the main enzyme involved in the digestion of these common vegetable lipids in the gastrointestinal tract. First in vitro studies were performed with medium chain synthetic galactolipids as substrates. We have then shown that recombinant guinea pig (Cavia porcellus) as well as human PLRP2 hydrolyzed at high rates natural DGDG and MGDG extracted from spinach leaves. Their specific activities were estimated by combining the pH-stat technique, thin layer chromatography coupled to scanning densitometry and gas chromatography. The optimum assay conditions for hydrolysis of these natural long chain galactolipids were investigated and the optimum bile salt to substrate ratio wa s found to be different from that established with synthetic medium chains MGDG and DGDG. Nevertheless the length of acyl chains and the nature of the galactosyl polar head of the galactolipid did not have major effects on the specific activities of PLRP2, which were found to be very high on both medium chain [1786±100 to 5420 ±85 U/mg] and long chain [1756±208 to 4167±167 U/mg] galactolipids. Fatty acid composition analysis of natural MGDG, DGDG and their lipolysis products revealed that PLRP2 only hydrolyzed one ester bond at the sn-1 position of galactolipids. PLRP2 might be used to produce lipid and free fatty acid fractions enriched in either 16:3 n−3 or 18:3 n−3 fatty acids, both found at high levels in galactolipids.

2. MALDI-TOF/MS analyses of cardiolipins

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Cardiolipin (CL) is a phospholipid located almost exclusively in energy transducing membranes such as the bacterial cytoplasmic membrane and the inner membrane of mitochondria [1]. CL is a dimeric phospholipid constituted by two phosphatidic acid molecules connected by a glycerol in the centre of the structure; therefore it contains four hydrophobic chains.

In the past cardiolipins have been analyzed mainly by HPLC and ESI/MS techniques. In particular in ESI/MS analyses CLs are typically detected as bi-charged species [M-2H]2-. It has also been demonstrated that cardiolipin can be analysed by MALDI-TOF/MS using 2,5-dihydroxybenzoic acid (DHB) and 2,4,6-trihydroxyacetophenone (THAP) as matrixes; more recently it has been shown that 9-aminoacridine (9-AA) is a suitable matrix for MALDI-TOF/MS analyses of phospholipids, resulting in a great increase in signal-to-noise ratio and resolution [2].

Here we have utilized matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) for the analysis of cardiolipins, using the novel matrix 9-AA. We have successfully analyzed different CL species isolated and purified from various prokaryotes (Salinibacter ruber, Salisaeta longa and Bacillus subtilis) as well as mitochondrial cardiolipin. Then we have successfully extended the use of MALDI-TOF/MS to the analysis of archaeal phospholipids and cardiolipins, isolated and purified by extremely halophilic microorganisms [3].

Finally we have developed a novel approach to obtain MALDI-MS membrane lipid profiles by direct analyses of lyophilized membranes, avoiding the steps of lipid extraction.

[1] E. Mileykovskaya, M. Zhang, W. Dowhan. 2004. Cardiolipin in Energy Transducing Membranes. Biochemistry (Moscow), Vol. 70, No. 2, pp. 154-158.

[2] G. Sun, K. Yang, Z. Zhao, S. Guan, X. Han, R. W. Gross. 2008. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometric Analysis of Cellular Glycerophospholipids Enabled by Multiplexed Solvent Dependent Analyte-Matrix Interactions. Analytical Chemistry, 80 (19), pp. 7576-7585.

[3] A. Corcelli. 2009. The cardiolipin analogues of Archaea. Biochimica et Biophysica Acta (BBA)-Biomembranes. Vol. 1788, pp. 2101-2106.

3. New Approaches of Artificial Lipid Vesicles and Bilayer Membrane's Modeling in Toxicological Studings

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Venoms produced by snakes of the family Viperidae contain proteins that interfere with the coagulation cascade, the normal haemostatic system and tissue repair, and human envenomations are often characterized by clotting disorders, hypofibrinogenemia and local tissue necrosis.

Studies on the interaction of snake venom and organized lipid interfaces have been conducted using a variety of systems, including BLMs, SUVs and LUVs. Giant unilamellar vesicles (GUVs) with a mean diameter of 30 μm have a minimum curvature and mimic cell membranes in this respect. GUVs were formed from the total lipid fraction from bovine brain by the electroformation method (Angelova and Dimitrov, 1987). *Vipera lebetina obtusa* venom was added to the sample chamber before the vesicles were formed. The membrane fluorescence probes, ANS and pyrene, were used to assess the state of the membrane and specifically mark the phospholipid domains. Fluorescent spectra were acquired on a *Varian* fluoremeter instrument.

ANS and pyrene allows us to quantify the fluidity changes in the membrane by measuring of the fluorescence intensity. The presence of viper venom in GUVs media reveals a noticable decreasing of membrane fluidity compare the control, while the binding of fluorophores with GUVs modified by venom lead to appearance of channel activity. It was recognized early (Sanchez & Bagatolli, 2002) that the vipers venom components preferred an organized lipid substrate near the lipid's phase transition and were particularly active against micellar lipids. These studies also emphasize the importance of a membrane surface curvature for its interaction with enzymatic components of venom.

4. Studying the interaction between lysophosphatidic acid and gelsolin using biophysical methods

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In signaling the activation of cell-surface receptors leads to selective gene transcriptions or immediate physiological reactions. In these processes many multicomponent complexes are formed at the membranes containing several proteins as well as membrane-lipids. The structure of signaling proteins is modular by which domain-domain or domain-lipid interactions are evolved. Some of these domains are responsible for lipid binding and are evolutionarily conserved among eukaryotes.

In eukaryotic cells lysophosphatidic acid (LPA), a phyisiologically important mediator of cell survival and migration, primarily acts on cell-surface receptors (edg-2, edg-4, edg-7) as a first messenger. However, it can also activate the nuclear receptor PPARgamma as well as affect membrane processes via mostly unidentified target proteins and mechanism. Recently, it was described that LPA interfares with the binding of phosphatidylinositol-(4,5)-bisphosphate (PIP2) to gelsolin, an actin filament severing protein containing a PIP2 binding PH (pleckstrin homology) domain. PH domains are widely spread among proteins that are involved in signaling pathways and regulating cell architecture of eukaryotic cells.

We have characterized the interaction between LPA and human gelsolin using biophysical and biochemical methods in vitro. We expressed and purified the cytosolic form of gelsolin and its PH domain, and also used the secretory form of the protein (plasma gelsolin). Based on our studies, the plasma as well as the cytosolic gelsolin binds LPA in micellar form through its PH domain. In titration experiments, the apparent Kd values are around the critical micelle concentration of LPA. The observed high cooperativity in the binding curves suggests that the PH domain as well as the intact proteins bind LPA in a clustered form - no binding occurs at low concentrations when LPA exists as monomers but strong binding emerges immediately when LPA clusters form. In the future we intend to figure out that LPA can bind to PH domain in a biologically relevant manner modulating the function of gelsolin, thereby affecting the spatiotemporal organization of the actin cytoskeleton.

5. #LIMP-2 sorting receptor of β-glucocerebrosidase: a cell-type specific mechanism

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Alterations in SCARB2 gene that codes for Lysosomal Integral Membrane Protein type 2 (LIMP-2), were recently described in patients with Action Myoclonus Renal Failure Syndrome (AMRF). AMRF is an autosomal recessive progressive myoclonic epilepsy without intellectual impairment associated with renal failure. We studied two Portuguese families where patients have consanguineous parents. In all the patients, the biochemical analysis revealed a normal ?-glucocerebrosidase activity in leukocytes, but a severe enzymatic deficiency in cultured skin fibroblasts. This deficiency suggested a defect in the intracellular sorting pathway of ?-glucocerebrosidase. The sequence analysis of the gene encoding LIMP-2, the sorting receptor for ?-glucocerebrosidase, confirmed this hypothesis, revealing a homozygous nonsense mutation in codon 178 (W178X). Besides lacking immunodetectable LIMP-2, patient fibroblasts also had decreased amounts of ?-glucocerebrosidase, which was mainly located in the endoplasmic reticulum, as assessed by its sensitivity to Endo H, which resulted in altered glycosilation patterns of ?-glucocerebrosidase. Differences in ?-glucocerebrosidase activity in leukocytes and fibroblasts, suggests that the mechanism of lysosomal sorting of ?-glucocerebrosidase is cell-type specific. We intend to call the attention to this rare disease that could contribute for the identification of undiagnosed patients and eventually to the deepening of the knowledge of the pathophysiology of this disorder.

6. LXR activation improves muscle insulin sensitivity in high-fat fed rats

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Liver X receptors (LXRs) are ligand-activated transcription factors of the nuclear receptor superfamily. LXRs have been characterized as key transcriptional regulators of lipid and carbohydrate metabolism. Synthetic LXR agonists were shown to stimulate lipogenesis via sterol regulatory element binding protein-1c leading to liver steatosis and hypertriglyceridemia. Despite their lipogenic action, LXR agonists show potent antidiabetic properties. LXR activation normalizes glycemia and improves insulin sensitivity in rodent models of type 2 diabetes and insulin resistance. Antidiabetic action of LXR agonists is thought to result predominantly from suppression of hepatic gluconeogenesis. LXRs are expressed also in skeletal muscle. However, it remains unclear whether LXR activation affects muscle insulin sensitivity. In the present study we attempted to answer this question.

The experiments were carried out on male Wistar rats fed for 5 weeks on either standard chow or high fat diet. The latter group was further divided into two subgroups receiving either selective LXR agonist - T0901317 (10mg/kg/d) or vehicle during the last week of the experiment. All animals were then anaesthetized and samples of the soleus as well as red (RG) and white (WG) sections of the gastrocnemius muscle were excised.

As expected administration of T0901317 to high-fat fed rats markedly augmented liver steatosis and hyperlipidemia. Nevertheless, it also normalized glucose tolerance and substantially improved whole body insulin sensitivity. This effect was accompanied by decreased expression of key genes of gluconeogenesis in the liver. In addition, LXR agonist rescued insulin-stimulated AS160 phosphorylation in all investigated muscles. Both mRNA and protein levels of muscle GLUT4 were also increased by the drug. Insulin-sensitizing effect of T0901317 was rather not related to changes in intramuscular level of lipid mediators of insulin resistance since neither diacylglycerol nor ceramide content was affected by the drug.

We conclude that improvement of muscle insulin sensitivity is one of the mechanisms underlying the antidiabetic action of LXR activators.

7. The role of lipids in the function and stability of two membrane proteins CXCR2 and MscL

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Membrane proteins constitute around 30% of all proteins within the cell and have become major drug targets for pharmaceutical companies. One major problem with in vitro protein studies can be their loss of stability and functionality which can lead to inaccurate in vivo drug testing. Lipids have already been proven to regulate the membrane proteins bR and DGK. One of the major problems encountered studying CXCR2, a G protein coupled receptor, in a Biacore is its' decrease in function over time and irreproducibility. By gaining an insight into how different solubilisation conditions and lipid environments can be exploited, the stability and functionality of CXCR2 can be optimised. Previous results have shown that lipids can be responsible for both specific and non specific protein regulation and hence are of utmost importance. Dynamic light scattering and X-Ray studies have highlighted the significance of ensuring equilibrated systems and uniform lipid phase behaviour . The more widely known protein MscL provides an excellent insight into the lipid effects on another membrane protein. Due to its' well characterised purification, this mechanosensitive channel is less complex to study in liposomes. By reconstituting purified MscL into liposomes of varying compositions the connections between bilayer changes or deformations and stability and function of the protein can be examined.

8. Squalene monooxygenase - a new promising target in a hypercholesterolemia therapy

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Hypercholesterolemia is a metabolic disorder characterized by elevated cholesterol level in serum. It concerns about 45% of the adult population and almost 10% of adolescents and is the main risk factor for heart disease and stroke.

Nowadays cholesterol-lowering therapy includes inhibitors of de novo cholesterol synthesis, cholesterol absorption inhibitors, bile acid resins, fibrates, and nicotinic acid. Unfortunately these drugs often severe adverse effects and what is more a large subset of patients does not respond to them.

Increasing global demand for new and innovative drug decreasing cholesterol level in serum prompted to the search of a new target for drug. Squalene monooxygenase, a key enzyme in cholesterol biosynthesis, seems to be the most promising one.

Squalene monooxygenase (SE) (EC 1.14.99.7) is a flavoprotein microsomal enzyme that catalyzes the conversion of squalene to (3S)2,3-oxidosqualene. Squalene accumulated due to SE inhibition is not toxic because it is a natural component of cell membranes. Even though a part of a squalene is converted into cholesterol, high squalene level has a positive effect on LDL and TG levels. Furthermore till now any adverse effects of SE inhibitors have not been reported.

This report presents following steps aim to human SE overexpressin: cloning human squalene monooxygenase gen into pET28sumo plasmid by SLIC technique, transformation of BL21-CodonPlus-RIL cells, transformants selection, optimalisation of protein expression, and protein identification by MALDI-TOF Mass Spectrometry.

The report demonstrates also one by one steps of squalene monooxygenase activity assay. It is based on SE isolated from rat liver and [3H] squalene which allows easy determination of reaction efficiently. Efficiency of this assay was also confirmed by identification of squalene and oxidosqualene by GC-MS. This enzymatic assay is the starting point to seeking squalene monooxygenase inhibitors.

9. Developing mass spectrometric methodology for assessing lipid metabolic flux

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Phosphatidylcholine (PC) is an abundant lipid component of eukaryotic cell membranes. Several metabolic pathways contribute to the synthesis and turnover of PC in cells. In Saccharomyces cerevisiae, PC can be synthesized by i) methylation of phosphatidylethanolamine (PE), ii) by the Kennedy pathway where choline is linked to diacylglycerol, or iii) by the condensation of lysoPC (LPC) and acyl-CoA. Albeit, the enzymes responsible for these metabolic transitions have been identified and characterised, only limited information is available on the kinetics of these reactions. Moreover, very little is known about how cells regulate the flux through these different metabolic routes in the context of the entire lipid metabolic network.

To address these questions we have devised a quantitative and comprehensive mass spectrometric approach for specifically monitoring PE, PC and intermediate monomethylphosphatidylethanolamine (MMPE) and dimethylphosphatidylethanolamine (DMPE) species. To this end, we employ high-resolution multiplexed tandem mass analysis of lipid precursors on a QSTAR mass spectrometer equipped with a robotic nanoflow ion source TriVersa NanoMate. Using growth media having a combination of stable-isotope labelled precursors (e.g. ethanolamineD4, cholineD9) and yeast strains harbouring single and double deletion of enzymes involved PE and PC metabolism we systematically investigated the flux of lipid metabolic transitions. This analysis confirmed previous observations on the structure-activity relationship of the enzymes mediating the PE methylation pathway (Boumann H. A., et. al., 2004 "The Yeast Phospholipid N-Methyltransferases Catalyzing the Synthesis of Phosphatidylcholine Preferentiall y Convert Di-C16:1 Substrates Both in Vivo and in Vitro*")Interestingly, our preliminary results also indicate a significant contribution of the transacylation route for producing molecular PE ad PE species with distinct fatty acid moieties.

10. #PI(3)P-dependent polymerization of actin propels phagosomes

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Actin polymerization drives the extension of pseudopods that trap and engulf phagocytic targets. The polymerized actin subsequently dissociates as the phagocytic vacuole seals and detaches from the plasma membrane. We found that phagosomes formed by engagement of integrins that serve as complement receptors (CR3) undergo secondary waves of actin polymerization, leading to the formation of "comet tails" that propel the vacuoles inside the cells. Actin tail formation was accompanied by, and required de novo formation of PI(3,4)P2 and PI(3,4,5)P3 on the phagosomal membrane. Class I phosphatidylinositol-3-kinases (PI3K) are not involved in the generation of the 3'-polyphosphoinositides because their substrate, PI(4,5)P2, was absent from sealed phagosomes. Instead, PI(3,4)P2 and PI(3,4,5)P3 are generated on the phagosomal membrane via phosphorylation of PI(3)P by phosphatidylinositol-4-phosphate 5-kinase.

Accordingly, inhibition of Vps34, the class III PI3K responsible for P I(3)P formation, prevented actin tail formation. These experiments reveal a new pathway leading to
PI(3,4)P2 and PI(3,4,5)P3 formation and signaling in endomembranes.

11. #Cholesterol levels determine AChR endocytic route in CHO-K1/A5 cells

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Stability of the nicotinic acetylcholine receptor (AChR) at the cell surface is critical to the correct functioning of the cholinergic synapse. Cholesterol (Chol) is an essential lipid that modulates AChR levels at the plasmalemma and ion translocation. We have studied the endocytosis of AChR in CHO-K1/A5 cells, a Chinese hamster ovary (CHO) cell line heterologously expressing murine muscle adult-type receptor under different Chol membrane content. Contrary to the norm, endocytosis of cell-surface AChR is accelerated by membrane Chol depletion via a hitherto unknown mechanism. This acceleration is no longer operative when membrane Chol levels are restored. We explored the possible mechanism involved in receptor loss in Chol-depleted cells (Chol-). Under such conditions the AChR is internalized by a ligand-, clathrin- and dynamin-independent mechanism, which does not involve the presence of the AChR-associated protein rapsyn. The small GTPase Rac1 is required: expression of a dominant negative form of Rac1, Rac1N17, abrogates receptor endocytosis. At variance with the endocytic pathway in control CHO cells, the accelerated AChR internalization proceeds even upon disruption of the actin cytoskeleton and does not depend on the cytoskeleton-associated inositol lipid PI(4,5)P2; its sequestration by the PH domain of phospholipase C does not alter endocytosis. AChR internalization under Chol- conditions is furthermore found to require the activity of Arf6 and its effectors Rac1 and phospholipase D. Thus, membrane Chol appears to act as a key homeostatic regulator of cell-surface receptor levels, determining the rate and mechanism of AChR endocytosis.

12. Characterization of lipids in skeletal muscles of inbreed mice lines: An ATR-FTIR spectroscopic study

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Purpose: Insulin resistance, which is the common property among many metabolic diseases, is the inability of insulin to exert its action on the periphery such as skeletal muscle and adipose tissue. The skeletal muscle plays an important role for glucose homeostasis and insulin resistance, thus is a corner stone in the development of type 2 diabetes mellitus and obesity. The underlying mechanism of the generation of skeletal muscle insulin resistance remains unclear. We hypothesize that intramyocellular lipid accumulation and different characteristics of lipids play a role in the generation of insulin resistance in skeletal muscle. This preliminary study aims to characterize the structural, functional and dynamical properties of lipids in Longismus dorsi (LD) muscles of one control (DBAJ) and eight different inbreed mice lines, four of which have been selected to have a high muscle content that constitute the lean lines (BMMICC866, BMMICC826, BMMINN806, BMMINN816 lines) and fo ur of them have been selected to have a high fat content that constitute the obese lines (BFMI852, BFMI856, BFMI860, BFMI861 lines). For this purpose Attenuated Total Reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was used. Among the inbred BMM lines that are used in this study, one has a known mutation (BMMICC line) and the other has an unknown genotype (BMMINN line). The ultimate aim of our study is to explain the relation between growth and obesity based on the macromolecular alterations.

Materials and methods: Longismus dorsi muscles from two different inbreed mice lines were directly placed on the ZnSe crystal of ATR-FTIR spectroscopy and scanned using in 4000-650 cm-1 region with a spectral resolution of 4 cm-1.

Results: As a result of this study, functional and structural characterization of lipids in eight different mice lines was performed. The content of saturated lipids was decreased in all groups except for BFMI861 line in comparison to control. However, the content of unsaturated lipids was found to be lower in BMM lines in comparison to control and BFM lines. Moreover, the frequency of the olefinic band shifted to lower values in all of the mice lines investigated, except for the BFMI860 line, in comparison to control. This results revealed that the unsaturated chains in the LD muscles of these lines are more ordered in comparison to control. The bandwidth of the CH2 asymmetric strecthing band, located at 2927 cm-1, decreased in BMMICC lines revealing the decrease in fluidity of membrane lipids. Moreover, the frequency of this band shifted to higher values in BMMICC lines, indicating that the membrane lipids were more disordered in comparison to the other lines. In addition, the length of the lipid acyl chains of BMMICC lines were found to be shorter in comparison to the other groups.

Conclusion: In this study, structural, dynamic and functional differences were observed in lipids of the investigated inbreed mice lines. We will further extend our analysis and will use some chemometric methods in order to characterize the macromolecular differences in these mice lines and to elucidate the relation between growth and obesity, based on the macromolecular alterations.

13. The ceramide transfer protein CERT as a regulator of life and death in glioma cells

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Ceramide (Cer), a key molecule in sphingolipid metabolism, is involved in signal transduction mechanisms that control cell growth arrest, differentiation and apoptosis. In particular, different studies demonstrate that in glial cells ceramide exerts antiproliferative and cytotoxic effects and strongly support that ceramide-signalling is altered in glial tumors. Due to the relevant effects of this bioactive lipid on cell fate, Cer-signalling is currently being studied as a target for novel strategies in cancer therapy. Generation and accumulation of ceramide in response to different cytotoxic treatments represent a widely studied cellular response to trigger growth arrest and cell death, while stimulation of sphingomyelin (SM) biosynthesis represents a pro-survival mechanism promoting the rapid consumption of Cer.

As the Cer transfer protein CERT is a key factor for the transport of Cer to the Golgi apparatus for SM biosynthesis, in this study we evaluated the possible role of CERT in the regulation of glioma cell fate. Although down regulation of CERT inhibited SM biosynthesis, we found that in glioma cells down regulation of CERT resulted in an increased cell proliferation associated to ERK1/2 hyperphosphorylation even in the absence of growth stimuli. In vitro assays, performed with homogenates obtained from CERT-dowregulated cells, indicated that the complex CERT/ceramide, but not CERT alone, is able to induce ERK1/2 dephosphorylation in a dose dependent manner. Immunoprecipitation experiments suggested a role of CERT as a scaffold protein for a phosphatase activity involved in Ceramide-dependent ERK1/2 inhibition.

Moreover, in CERT-silenced cells, ceramide is no longer able to inhibit the mitogenic effect of different growth stimuli indicating that CERT is crucial for Cer-mediate d effects on cell growth inhibition. In addition, we found that CERT is not only involved in the antiproliferative activity of ceramide but can also participate to the citotoxic effect of this mediator, as in CERT-downregulated glioma cells the administration of exogenous ceramide failed to induce cell death.

Taken together these results strongly suggest a pivotal role of CERT for the interaction of ceramide with downstream targets that control glioma cell proliferation and death.

14. MAT1A-KO mice exhibit distinctive mechanisms underlying the imbalanced triglyceride metabolism during the origin and development of non-alcoholic fatty liver disease

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Background/Aims: Methionine adenosyltransferase 1A gene (MAT1A) is expressed mainly in mature liver and catalyzes the synthesis of S-adenosylmethionine (SAM), the main methyl donor in some biological processes, such as the formation of phosphatidylcholine (PC) from phosphatidylethanolamine (PE). At 3 months (3-KO), mice lacking MAT1A are more susceptible to a choline deficient diet-induced fatty liver, at 5 months develop steatosis spontaneously, which progresses to non-alcoholic steatohepatitis (NASH) at 8 months (8-KO), and may degenerate into hepatocellular carcinoma at 16 months. We have reported that hepatic TG secretion decreases in 3-KO mice and, in contrast, it increases in 8-KO mice. Interestingly, both MAT1A-KO mice displayed an overproduction of smaller VLDL. Therefore, our aims were to find out the mechanisms that lead to a disrupted VLDL assembly and secretion and their effects over the TG distribution among plasma lipoproteins in these two stages of non-alcoholi c fatty liver disease (NAFLD) due to SAM deficiency.

Methods: Liver microsomes were prepared by serial centrifugations and lipids were extracted, separated, charred and densitometrically quantified. Triglyceride lipase (TGL) and diacylglycerol acyltransferase (DGAT) activities were determined by monitoring the metabolism of [14C] among different substrates. MTP TG transfer activity was measured using a fluorescence assay. The hepatic lumen and membrane apoB content in microsomes was measured by western blotting. Hepatic gene expression was determined by real-time RT-PCR. Plasma lipoprotein subclasses were isolated by size-exclusion HPLC and TG was analyzed with enzymatic kits.

Results: 3-KO mice had decreased TGL and DGAT activity in microsomes concomitant with reduced FFA levels in microsomes and plasma in comparison with their WT littermates. In addition, apoB was retained in the microsomal lumen of 3-KO mice and its gene expression was not altered in MAT1A-KO mice. The gene expression of TGL and AADA was down-regulated in pre-steatotic and NASH mice but no changes were observed in the levels of DGAT-2 mRNA. MTP activity rose in parallel with liver and microsomal TG content in 8-KO mice, suggesting the transference of TG into the endoplasmic reticulum lumen during VLDL-apoB assembly. Besides, a decreased microsomal PC/PE ratio was observed in this stage of NAFLD. As a possible consequence of the described events, MAT1A-KO mice showed an altered plasma lipoprotein profile, with increased IDL/LDL-TG at 3 months, and raised small LDL-TG at 8 months.

Conclusion: SAM-deficiency and subsequent impaired VLDL assembly and secretion in MAT1A-KO mice in a stage prior to hepatic steatosis and in another with steatohepatitis respond to distinctive mechanisms and might be a mandatory step in the NAFLD associated dislypemia.

15. Differentiated Placenta-Derived Mesenchymal Stem Cells as Models for Pulmonary Surfactant Biogenesis and Trafficking

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Pulmonary surfactant is a lipid-protein complex that lowers surface tension at the air/liquid interface of the alveoli, preventing collapse of the lung on expiration and stabilizing the respiratory surface along compression-expansion breathing cycles. Surfactant mass is comprised of approximately 90 % lipids and 8-10 % of some specific proteins. Phospholipids (80% surfactant mass) , mainly dipalmitoylphosphatidylcholine (40%) and phosphatidylglycerol (10-15%) are the main surface active agents, but presence of hydrophobic surfactant proteins SP-B and SP-C is strictly required to facilitate phospholipid dynamics during surface film formation and stabilization. Lack or dysfunction of pulmonary surfactant is associated with severe respiratory pathologies such as neonatal respiratory distress syndrome in preterm infants or acute respiratory distress (ARDS) associated with lung injury.

Surfactant is synthesized and secreted by type II pneumocytes (alveolar type II cells) in the alveolar epithelium. Established surfactant-producing pneumocyte-like cell-cultured lines are still not available, preventing a proper and detailed characterization of biosynthetic and trafficking pathways involved in surfactant biogenesis. Recently, differentiation of placenta-derived mesenchymal stem cells to cells with the phenotypic features of pneumocytes (PLCs, pneumocyte-like cells) has been achieved. The present study has approached morphological, ultrastructural and biochemical characterization of these PLCs, including analysis of the expression pattern of surfactant-related specific proteins, such as surfactant proteins SP-A, SP-B and SP-C and the lipid transporter protein ABCA3 (ATP-binding cassette A3). ABCA3 is localized at the limiting membrane of lamellar bodies (LBs), the organelles in charge of storing and secreting surfactant, and is proposed to function as a lung s urfactant lipid importer, implicated in the selective accumulation of surfactant lipids into the highly packed structures of LBs. Placenta-derived PLCs could therefore provide an useful ex vivo model for the study of surfactant biogenesis, including characterization of protein processing and lipid traffic, as well as the packing and storage of surfactant lipid-protein complexes into LBs.

16. Role of lipid droplets in ERAD

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Lipid droplets are intracellular and cytoplasmic structures with role in lipid storage, having a neutral lipids core and a surface monolayer of polar lipids with attached or embedded proteins. The mechanism of lipid droplets formation is not known, but they are often associated with the endoplasmic reticulum membrane and display perinuclear localization.

At least one protein, ApoB is recruited by lipid droplets and targeted to degradation in proteasomes, suggesting that this maybe a mechanism used by other incompletely folded proteins. Misfolded proteins expose hydrophobic residues and tend to aggregate, being very toxic to the cell and in this case the droplet core might provide an appropriate environment for the hydrophobic patches, preventing aggregation.

Misfolded ER proteins are degradated by ERAD (ER-associated degradation): retrotranslocated from the ER, marked with polyubiquitin and degraded in proteasome. The retrotranslocation mechanism is not elucidated, and one proposed mechanism is the vesicular traffic to the proteasome possibly by lipid droplets.

My PhD projects aims to investigate the traffic of an ERAD substrate from the ER to the proteasome and the efficiency of its presentation to T cells. In this order we want to see if lipid droplets are involved in transport from ER to the proteasome of misfolded proteins targeted for proteasomal destruction using as model soluble tyrosinase (ST), lacking the transmembrane domain and cytosolic tail.

17. Role of PI4KIII in lipid signaling during cold exposure in Arabidopsis thaliana.

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In eukaryotic cells, phosphoinositides such as phosphatidylinositol 4-phosphate (PI4P) and phosphatidylinositol (4,5) bisphosphate (PI(4,5)P2) are mediators of a number of intracellular events, ranging from signal transduction to cytoskeletal rearrangements and membrane trafficking. Phosphatidylinositol 4-kinase (PI4K), the enzyme that synthesizes PI4P, is the first committed step in their biosynthesis, therefore it could represent a key regulation point of such events. We investigated the role of PI4K in cold response in the Arabidopsis thaliana plant model. As it was previously shown in the lab, a short cold exposure of Arabidopsis cells induces a phospholipase C activation, mediating important transcriptome changes. In plant, as in other organisms, PLC catalyses the production of second messengers by hydrolysing PI(4,5)P2, synthesized by PI4P phosphorylation. We demonstrated that PI4K display a role in cold signalisation in Arabidopsis plantlets. Thanks to both pharmaco logical and genetic approaches, we revealed which of the various PI4K isoforms are involved in supplying the PLC with its substrate during cold activation. By monitoring both phosphoinositides and transcriptome changes, as well as PI4K mutants phenotypes at low temperature, we highlighted differential roles for the various PI4K in cold response.

18. A nutrigenomic approach reveals that LXR is required for hepatic steatosis induced by essential fatty acid deficiency

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In mammals, dietary essential polyunsaturated fatty acids (PUFAs) influence the expression of genes involved in de novo hepatic fatty acid biosynthesis (lipogenesis). A deficiency in dietary PUFAs results in increased expression of lipogenic enzymes in the liver and ultimately in aberrant triglycerides (TGs) accumulation (hepatic steatosis). The Liver X Receptor (LXR) is a nuclear receptor that plays a part in the transcriptional control of lipogenesis. The hypothesis that LXR is involved in mediating the effects of dietary fatty acids on lipogenesis has been proposed but remains controversial.

We used a nutrigenomic approach to investigate the role of LXR in the hepatic response to dietary lipids. We used wild-type (W-T mice) and transgenic mice lacking both a and b isoforms of LXR (LXR-/- mice). These adult male mice were fed for nine weeks with three isocaloric diets containing similar amount of fatty acids. However, the three diets contained no PUFAs, standard PUFA proportion, or a high PUFA proportion.

In mice of both genotypes the effect of the diets on hepatic PUFA levels was assessed through fatty acid profiling. We observed that the deficiency in dietary PUFAs induced an expected steatosis paralleled with a marked increase in lipogenic genes expression in W-T mice but not in LXR-/- mice. These data highlight for the first time the role of LXR in the response of hepatic lipogenesis to dietary PUFAs. Interestingly, we observed that dietary PUFAs markedly influence the expression of enzymes involved in both cholesterol and oxysterol metabolism. Oxysterols are described as natural activating ligands for LXR. Therefore, we postulated that the marked regulation of enzymes involved in oxysterol synthesis that occurs in response to dietary PUFAs may influence lipogenesis via LXR.

19. Role of MLCL AT-1 in Cardiolipin Metabolism: A potential therapeutic approach to Barth Syndrome

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Barth Syndrome (BTHS) is a rare X-linked genetic disorder that is caused by a mutation in the Tafazzin (taz) gene. Tafazzin is a transacylase enzyme localized in the outer membrane of mitochondria and is involved in the biosynthetic remodelling pathway of cardiolipin (CL), a lipid necessary in the complex that drives the oxidative process in the mitochondria. BTHS is believed to be the only genetic disease linked to a biochemical deficiency of CL and accumulation of its precursor-monolysocardiolipin (MLCL). We investigated the contribution of another CL remodelling enzyme, monolysocardiolipin acyltransferase-1 (MLCL AT-1), to the remodelling of CL in normal human lymphoblasts and in lymphoblasts from BTHS patients. MLCL AT-1 or taz were knocked down in normal human lymphoblasts using RNAi and MLCL AT-1 enzyme activity examined in human lymphoblasts. MLCL AT-1 enzyme activity was reduced approximately 50% ($p<0.05$) when MLCL AT-1 was knocked down in normal human lymphoblasts.

In contrast, MLCL AT-1 enzyme activity was unaltered when taz was knocked down. Knock down of both MLCL AT-1 and taz simultaneously did not result in a further reduction in MLCL AT activity compared to knock down of MLCL AT-1 alone. These studies indicate that MLCL AT-1 may act independently of taz in the remodelling of CL. Expression of MLCL AT-1 in BTHS lymphoblasts with different mutations in taz elevated CL levels over 2-fold ($p<0.05$) in these cells compared to mock transfected control cells. These studies indicate that expression of MLCL AT-1 may elevate CL in all BTHS lymphoblasts regardless of the mutation in taz.

20. Interacting partners of a phosphatidylinositol transfer protein as an indicator of protein function.

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The mammalian genome encodes three soluble phosphatidylinositol transfer proteins (PI-TP), PITP?, PITP?, and the less well-characterized RdgB?. PITP? is essential in neurons for axonal outgrowth, and mice lacking PITP? suffer from neuronal degeneration (vibrator mice). The PITPs transfer phosphatidylinositol (PI) in exchange for phosphatidylcholine (PC) between membranes. Delivery of PI by PITP? provides the substrate for the enzymes phospholipase C and PI 3-kinase, PI(4,5)P2, at the tip of the developing axon. PITP? possesses an essential house-keeping role in cells as loss of both PITP? alleles by genetic ablation in murine embryonic stem cells is lethal. Such activities include a role in cytokinesis and the regulation of retrograde traffic from the Golgi to the ER. Considering PITP? and PITP? have such important roles in mammalian cells, what is the role of the third soluble PITP, RdgB?? RdgB? was first cloned in 1999 (Fullwood et al). A second splice variant (sp2) which differs from the first (sp1) in the sequence of its C-terminal tail, was identified in 2003 (Takano et al). Using PI and PC transfer assays and [14-C] lipid binding assays, we have observed that RdgB? binds PI and PC in different ratios to that seen for PITP? and PITP?. Furthermore, RdgB? binds a third, as of yet unidentified lipid with a similar affinity to that which it shows towards PI. A yeast two-hybrid genome-wide screen for interacting partners has indicated that RdgB? sp2 interacts with the angiotensin II receptor-interacting protein ATRAP; a protein which, when over-expressed, protects against cardiac hypertrophy. Our immunoprecipitation experiments indicate other binding partners for RdgB?. Identification of these interacting proteins together with the 'mystery' lipid will give vital clues as to the function of RdgB?

21. Phosphatidylethanolamine, a Key Lipid of Mitochondria

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Phosphatidylethanolamine (PE) is essential for many types of cells. In the yeast Saccharomyces cerevisiae PE is one of the major phospholipids in membranes, where it contributes to membrane function and integrity. Yeast PE synthesis is accomplished by a network of reactions which comprise four different pathways: (I) decarboxylation of phosphatidylserine (PS) by the mitochondrial phosphatidyl-serine decarboxylase1 Psd1p, or (II) by Psd2p in a Golgi/vacuolar compartment; (III) the so-called CDP-ethanolamine pathway in the endoplasmic reticulum (ER) which utilizes ethanolamine as a substrate; and (IV) acylation of lyso-PE catalyzed by the acyl-CoA-dependent acyltransferase Ale1p in the mitochondria associated endoplasmic reticulum membrane (MAM). The enzyme which contributes most to PE formation in the yeast is Psd1p localized to the inner mitochondrial membrane. To obtain a general view of the role of PE in the cell and to study the effects of an unbalanced PE level, a psd1? deletion mutant was subjected to DNA-microarray. This study demonstrated that a number of genes were up-regulated in a psd1? deletion strain compared to wild type. To obtain more insight into the physiological function of the respective gene products, we analyzed the growth phenotype on different carbon sources of yeast mutants bearing defects in the respective genes and the phospholipid profile of these strains. These investigations revealed that among the candidate strains three mutants (rtc2?, gpm2?, gph1?) exhibited growth defects on fermentable and non fermentable carbon sources. We also detected changes in the PE level of homogenate and mitochondria. We assume that genetic and/or functional interaction of these genes/gene products with PSD1 may occur.

To address this question, double mutants with psd1? were constructed which are currently under investigation.

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22. Role of LPP3 in the structural and functional organization of the Golgi complex

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Diacylglycerol is necessary for trans-Golgi network (TGN) to cell surface transport, and also for the formation of COP-I vesicles in the Golgi-to-ER transport pathway. Diacylglycerol is produced by several metabolic pathways. Lipid phosphate phosphatases (LPPs) also known as phosphatidic acid phosphohydrolases (PAP), for example, produce DAG through the hydrolysis of phophatidic acid. LPP3 is a member of this family localized in the Golgi complex. Our goal is to demonstrate that LPP3 is involved in the production of DAG at the Golgi level and also to characterize the role of this DAG in the Golgi structure and function.

To carry out this work we have inhibited the expression of LPP3 in swiss3T3 cells using specific shRNA's introduced by lentiviral infection, getting a 50-70% decrease in LPP3 levels.

In swiss3T3 cells silenced for the expression of LPP3 we have observed a delocalization of the PKCθ C1 domain from the Golgi membranes, indicating a decrease in DAG levels. In transport experiments, using transport carriers such as the Shiga toxin molecule, which fused to the KDEL sequence is internalized from the cell surface to the ER passing through the Golgi complex, we have observed a delay in the Golgi to ER transport in those cells silenced. Finally, using electronic microscopy we have characterized alterations in the ultra structure of the Golgi complex in those cells which have been silenced for LPP3, observing an increase in the number of COP-I vesicles attached to the Golgi membranes, indicative of an incomplete fission process.

All of these results let us conclude that LPP3 participates in the DAG homeostasis at the Golgi level, and in the COP-I vesicle formation in the Golgi-to-ER transport pathway.

23. The Fate of Fatty Acids

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Obesity occurs mainly in developed countries due to high fat diet and can be associated with diseases as nonalcoholic steatohepatitis, insulin resistence, Type II Diabetes mellitus and cardiomyopathy. Obese people accumulate lipids not only in adipose but also in non adipose tissue. Therefore the term lipotoxicity was introduced indicating the involvement of lipids in mediating the toxic effect. However the underlying molecular mechanism remains unclear. There is evidence that not triacylgylcerols (TAG) accumulation itself is responsible for lipotoxicity, but fatty acids (FA) and diacylglycerols play also a prominent role.

To begin to understand the molecular mechanism of lipotoxicity, we have first investigated the interconnection between FA de novo synthesis, TAG synthesis and PL synthesis. Therefore a mutant with constitutively upregulated FA de novo synthesis was generated (acc1*) to investigate the effect of chronic FA supply on lipid homeostasis. As a consequence of this deregulated FA de novo synthesis TAG massively accumulate in lipid droplets; however, the cells are vital and grow as the wild type, in the presence of inositol. We conclude that TAG synthesis serves as a buffer to detoxify excess FA under this conditions. Next, we generated a triple mutant lacking the main TAG forming enzymes Dga1p and Lro1p, in combination with the hyperactive acc1* mutant. Despite upregulated FA synthesis, this triple mutant that is devoid of the major diacylglycerol acyltransferases lacks lipid droplets and TAG altogether, however, it displays massive proliferation of intracellular membrane stacks. Fu rthermore, the vitality of the cells is strongly reduced, indicating a lipotoxic effect of excess endogenous fatty acids that are preferentially channeled into PL, in the absence of TAG synthesis. Recent data of the lipotoxic effect of lipids will be presented.

24. Involvement of the phospholipase D/ mTOR pathway in the regulation of muscle cell size

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Muscle atrophy is a major feature of many physiological or pathological situations ranging from aging to immobilization, space flights, starvation, diabetes, immunodeficiency and cancer. The protein kinase mTOR, which integrates signals originating from growth factors, nutrients supply, energy availability, mechanical stimuli, is a key regulator of muscle tissue homeostasy, controlling the rates of protein synthesis and degradation, and the size of myocytes.The importance of phospholipase D (PLD) and its product phosphatidic acid (PA) as regulators of mTOR activity has recently been recognized. PA binds to, and activates, mTOR protein. We have previously shown that PLD activity is required for differentiation of myogenic cells in culture, and that this involves mTOR activation. We presently address the question of the involvement of the PLD/mTOR pathway in the control of muscle tissue mass.

In differentiated rat L6-myotubes, the specific inhibition of PA production by the primary alcohol Butanol-1 induced muscle cell atrophy and an inhibition of mTOR, as reflected by a decreased phosphorylation of its substrates. Using siRNA approaches we demonstrated that PLD1 isoform knockdown induced myotubes atrophy and abrogated mTOR activity, while knockdown of PLD2 isoform had little effect. To confirm the role of PLD and particularly PLD1 in the control of myocyte size, we overexpressed either PLD1 or PLD2 isoform, by using adenovirus constructs. Whereas overexpression of PLD2 isoform was without effect on myotube size, PLD1 overexpression induced myotube hypertrophy and increased mTOR activity. Furthermore, addition of PA, or PLD1 overexpression counteracted the atrophic effects of dexamethasone.

These results show a new potential role for PLD in the maintenance of muscle size, through the stimulation of mTOR. The demonstration of an anti-atrophic role of PLD and PA could suggest new therapeutic approaches to limit the deleterious effects of muscle wasting, e.g. the use of stable PA analogues.

25. #Sphingomyelin analogues with differently branched N-acyl chains: effect on acyl chain order and sterol interaction in bilayer membranes

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Sphingolipids are found in the external leaflet of plasma membranes and are considered to be structurally and functionally essential component of eukaryotic cells. Sphingolipids play important roles in lateral domain formation in biological membranes and helps in cell recognition and signalling. Natural sphingolipids display an extensive diversity in their structures both in the head group and hydrophobic backbone. Although sphingosine (D-erythro-2-amino-trans-4-octadecene-1,3-diol) is the prevalent backbone in most mammalian sphingolipids, including sphingomyelin, also branched and unsaturated long chain exists in for example beef and rat kidneys. It has been clearly shown that both the long chain base and acyl chain configuration has marked effects on the molecular properties of sphingomyelins. However, the functional role of methyl-brancing in the long chain base or in the N-linked acyl chains of sphingomyelins is not fully understood. Methyl-branching can be envisioned t o interfere with bilayer packing of sphingolipids, since the protruding methyl group is likely to attenuate attractive van der Waals forces among interacting acyl chains.

It is the aim of the present study to examine how methyl-branching in the N-linked acyl chain of sphingomyelins affect their membrane properties as compared to non-branched analogues. We have syntesized sphingomyelin analogues with methyl groups at different positions along the acyl chain including a sphingomyelin analogue with N-linked phytanic acid. Interactions between branched sphingomyelin analogues and unbranched sphingomyelin (N-palmitoyl sphingomyelin and N-stearoyl sphingomyelin) was examined with differential scanning calorimetry, whereas lateral domain formation and sterol interaction by branched sphingomyelin analogues was determined using fluorescence quenching and a sterol partitioing assay. Our results show that methyl-branching and the poistioning of the methyl group markedly interfered with molecular packing of the sphingomyelin analogues.

26. #Linking Lipid Binding to Gluose Metabolism: Increased Insulin Sensitivity in Mice with Homozygous Disruption of Thioesterase Superfamily Member (Them) 2

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Background: Them2 is a mitochondrial acyl-CoA thioesterase, which we have identified as a PC-TP-interacting protein. PC-TP stimulates Them2 activity in vitro, but the biological implications are unclear. Aim: Because mice lacking PC-TP (Pctp-/-) exhibit marked increases in hepatic insulin sensitivity, the aim of this study was to examine a role for Them2 in regulating insulin sensitivity in the liver. Methods: We developed Them2-/- mice, which lack 2 of the 3 exons that encode Them2. Them2-/- mice lacked detectable expression of both Them2 mRNA and protein. Plasma glucose concentrations of chow fed Them2-/- mice (n=9) and wild type littermate controls (wild type, n=7) were measured following an overnight fast and then periodically up to 180 min after i.p. injection of insulin (1 U/kg bw), pyruvate (2 mg/g bw) or glucose (1 mg/g bw) for tolerance tests to insulin (ITT), pyruvate (PTT) or glucose (GTT), respectively. Plasma insulin was measured by ELISA. Gene expression was de termined by quantitative real-time PCR. Results: Them2-/- mice developed normally and did not exhibit any overt abnormal physical characteristics. Body weights (g, mean±SEM) of Them2-/- mice were slightly lower than wild type mice (wild type, 30.5±1.0; Them2-/-, 27.9±0.8, $p<0.04$). Fasting plasma glucose and insulin concentrations were unchanged in the absence of Them2. Indicative of increased clearance of exogenous glucose, plasma glucose concentrations in Them2-/- mice were lower at each time point during the GTT and the area under the plasma glucose concentration curve (AUC) was reduced 27%. Consistent with increased hepatic insulin sensitivity, the AUC for the PTT, which is a measure of hepatic glucose production, was reduced 45% in Them2-/- mice. The ITT, an indicator of whole body insulin sensitivity, did not differ between Them2-/- and wild type mice. Consistent with decreased hepatic glucose production, the mRNA expression of PEPCK was reduced in the liver of Them2 -/- mice, as was FOXO1, HNF4α and PGC1α, which are the transcript.

27. Atmospheric pressure photoionization mass spectrometry as the valuable method for identification of polyisoprenoid alcohols in natural samples

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Polyisoprenoid alcohols are linear five-carbon unit polymers which occur in all living cells. Their chains are built of 5-100 and more isoprenoid units creating two groups of these compounds according to the structure: dolichols and polyprenols. Dolichols, mainly found in animal tissues, yeast cells and some plant organs like roots, are ? – saturated isoprenoids alcohols. Polyprenols occurring in plant photosynthetic tissues, wood, seeds, flowers and in bacterial cells are ? – unsaturated isoprenoids alcohols.

These compounds occur mainly as a free alcohols or carboxylic esters in living organisms. The biological role of dolichols and polyprenols is still examined. Free polyisoprenoid alcohols or their carboxylic esters have been postulated to modulate, as a structural component, the physical properties of model membranes. Yeast-specific polyisoprenoid alcohols are postulated to take part in the transport of ER and vacuolar proteins. Dolichyl phosphates are considered to serve as cofactors in protein glycosylation and biosynthesis of glucosyl phosphatidyl inositol anchor in animal and probably also in plant cells. Protection of cellular membranes against peroxidation is a new role of dolichols which has been suggested recently [1, 2].

Dolichols and polyprenols are always identified as the mixtures of prenologues in cells. HPLC-MS is a useful method to separate and analyze the mixtures of polyisoprenoid alcohols. Electrospray ionization (ESI) is popular ionization method to identify polyprenols and dolichols by mass spectrometry coupled with liquid chromatography.

In this study, atmospheric pressure photoionization (APPI) method was examined to analyze polyprenols and dolichols. The results were compared with results obtained by electrospray ionization technique.

The ionization process in APPI is initiated by 10 eV photons, emitted by a krypton discharge lamp [3]. The investigations show that it is a sufficient energy for ionization of dolichols and polyprenols. Using of dopant is not required in the measurements. In contrast to electrospray source, application of APPI technique permits an effective ionization of dolichols and polyprenols in polar (i.e. methanol) and nonpolar (i.e. hexane) solvents. It gives an opportunity to analyze mixtures of polyisoprenoid alcohols by normal and reverse phase liquid chromatography coupled with mass spectrometry. Additionally, the smaller concentrations of analytes are required. Identification of dolichols and polyprenols by APPI MS can be carried out in positive and negative ion mode. In APPI mass spectra, protonated dolichols ions are present in positive ion mode while APPI mass spectra of polyprenols are dominated by peaks corresponding to protonation and immediate loss of water from the proton ated molecules. In negative ion mode [M+O2].- and [M+Cl]- ions are observed in mass spectra of both analyzed compounds.

APPI source seems to be a method of choice for successfully identification of isoprenoid alcohols in natural mixtures.

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28. #Effect of cholesterol-lowering drugs on human ABCA1 cell surface expression

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The ABCA1 protein plays a pivotal role in reverse cholesterol transport, by mediating the generation of HDL particles and removing cellular cholesterol. Mutations in ABCA1 cause Tangier disease, a disorder characterized by very low HDL levels, cholesterol deposition in macrophages, and premature atherosclerosis. Subcellular localization of ABCA1 bears particular importance, since both the proper expression in the plasma membrane and internalization along with apoA-I are required for correct function of ABCA1.

In the present study we demonstrate a quantitative in vitro test system, which is suitable for monitoring the plasma membrane level of ABCA1. We introduced a hemagglutinin (HA) epitope into the first extracellular loop of the functional and non-functional ABCA1 variants, and stably expressed them in various mammalian cell lines. After characterization of the expression level, proper localization and function of different ABCA1 variants, we followed quantitatively their cell surface expression by immunofluorescence staining, using flow cytometry. Using different substances which are known to influence ABCA1 trafficking and degradation, we demonstrated the applicability and reliability of our cellular test system.

Next we studied the effect of several cholesterol level-lowering drugs and ABCA1 inhibitors on the cell surface expression of ABCA1. Interestingly, we found that ezetimibe, a blocker of the intestinal cholesterol absorption, reduces ABCA1 cell surface expression only in the case of a functional ABCA1.

Our model system provides a new tool for acquiring more information on the post-translational regulation, internalization, degradation and recycling of the ABCA1 protein.

29. Different biological properties affecting the glycolipid transfer protein

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The glycolipid transfer protein (GLTP) is a ubiquitous, cytosolic protein that selectively mediates the transfer of glycolipids between lipid membranes in vitro. The biochemical properties of GLTP are well known, but the precise biological function of the protein remains elusive. The GLTP structure is unique from other lipid binding/transferring proteins; hence the protein is now considered as a prototype and founding member for a new GLTP superfamily. It is likely that GLTP is involved in events on the cytosolic side of the plasma membrane or the endoplasmatic reticulum (ER), possibly functioning as a carrier of glycolipids or as an intracellular sensor of glycolipid levels. Some studies suggest that GLTP may be involved in the intracellular translocation/sensoring of glucosylceramide in particular. By pursuing various research methods we intend to shed more light on the biological role of GLTP.

30. Cerebrospinal fluid lipidomics in Alzheimer's disease

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Many neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are associated with alteration of lipid metabolism (Wenk 2005, Abidhatla et al. 2006, Postle 2009). Despite the fact that evidences of lipid changes occurring in brain of AD patients have been published, only a few attempted to characterize the lipid profile of cerebrospinal fluid and dissect the potential differences in AD patients. Cerebrospinal fluid (CSF) is the most informative fluid source for neurodegenerative disease prognosis, due to constant physical contact with brain and is readily obtainable compared to tissues. Analysis of phospholipids and other lipids in CSF offers a high potential for lipid biomarker discovery, in neurological diseases (Han and Gross 2003, Postle 2009) and for understanding essential aspects of associated molecular processes.

In this work we developed a high-throughput and sensitive method based on nanoHPLC/ESI QTOF MS for phospholipid screening in the CSF. We show that from a crude lipid extract (equivalent to 0.4 µl of CSF) we can easily detect and quantify different phosphatidylethanolamine, phosphatidylcholine, phosphatidlyinositol and sphingomyelin species. The developed method was used in a differential display study involving CSF samples of AD patients and non-demented controls, to conclude on its potential application in lipid biomarker discovery.

31. #Identification and investigation of novel proteins involved in lipid droplet morphology and protein targeting in yeast

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Lipid droplets (LD) are the storage organelles for neutral lipids (triacylglycerols and steryl esters) in almost all eukaryotic cells. Since the major activities of neutral lipid biosynthesis and -breakdown take place on the LD, many diseases are potentially linked to defective enzymes involved in their biogenesis. We use the baker´s yeast Saccharomyces cerevisiae, a eukaryotic single cell organism, to identify factors involved in neutral lipid homeostasis and LD assembly. The relatively small genome of some 6000 genes and the facileness of biochemical and genetic manipulation makes it a promising model system to understand physiological and pathological processes involved that may also be relevant for mammalian cell types. Specifically, we aim at identifying the mechanisms involved in protein targeting to LD, which is currently unknown and subject to extensive investigation in numerous cellular systems.

To identify novel proteins involved in LD biogenesis, morphology, homeostasis and targeting, we performed an imaging-based screen of the yeast deletion mutant collection. Two strains were constructed, each expressing from a chromosomal locus an LD-resident protein tagged with a Green Fluorescent Protein, namely Faa4-mGFP, an acyl-CoA synthetase localizing to LD and the endoplasmic reticulum, and mGFP-Tgl4, a triacylglycerol lipase that localizes exclusively to the LD. These „query strains“ were crossed against the entire collection of more than 4700 viable single knockout mutants, using the synthetic genetic array method (Tong, Boone 2006). With this method, a desired marker or mutation is integrated in a systematic manner into an array of mutant strains. After the selection of haploids carrying both the fluorescent reporter and a single gene knockout, the mutants were imaged using high resolution confocal fluorescence microscopy, and mutant cells were characterized regarding size and number of LD, or mislocalization of the GFP-signal.

This analysis yielded more than 200 strains which showed a strongly deviant LD phenotype. Notably, only few overlapping hits were identified in both screens, and there was also little overlap with two similar screens, in which LD were stained with vital dyes, Nile red or BODIPY (Szymanski, Binns et al. 2007; Fei, Shui et al. 2008). These observations confirm the highly dynamic behavior of LD and neutral lipid metabolism depending on growth and experimental conditions, and further suggest that these screens are far from being saturated. Our analysis confirmed several of the candidate proteins previously described, specifically the yeast Fld1p, a homolog of mammalian seipin (BSCL2) that is involved in a severe disease termed Berardinelli-Seip Congenital Lipodystrophy Type 2, the yeast AMP-activated protein kinase, Snf1p, and the phosphatase subunits Nem1p and Spo7p that regulate phosphorylation of the yeast lipin, the phosphatidic acid phosphatase Pah1p. Surprisingly, most significant hits in our screen were in the categories chromatin remodeling, protein transport and protein phosphorylation. These findings support the notion that triacylglycerol- and LD-homeostasis are connected to multiple cellular processes and metabolic pathways, which are now subject to further biochemical analysis.

One of the identified factors specifically affects the subcellular localization of the test construct, Faa4-mGFP. Despite unchanged neutral lipid content and wild type LD morphology, Faa4-mGFP fails to localize correctly to the LD and rather remains in the ER and cytosol. This effect was not observed with other ER/LD associated proteins, underscoring the specificity of this activity. This is the first description of a "scaffolding" protein required for targeting or sequestering a protein to the LD in yeast. The specificity and functional role of this process are currently under further investigation.

32. System level approach reveals that a putative methyltransferase is required for lipid homeostasis during stress imposed by cantharidin in Saccharomyces cerevisiae

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Understanding the mechanisms of unwanted toxicity of clinically relevant compounds will help to design more effective therapies to treat human diseases. Using system-level approach in Saccharomyces cerevisiae we demonstrated how cytotoxicity to cantharidin, a potent inhibitor of protein phosphatases, is mediated by previously uncharacterized SAM-dependent methyltransferase CRG1. CRG1 was identified as gene dose-dependent regulator of resistance to cantharidin. To further investigate the molecular mechanism of CRG1-dependent cantharidin toxicity we integrated the genome-wide screens (Synthetic Genetic Array, profile of double deletion mutant fitness with the drug, expression analysis) combined with comparative lipidome analysis by mass spectrometry. The systematic phenotypic analysis and the leads from the genome-wide screens revealed that CRG1 functionally interacts with lipid-related processes. Furthermore, the analysis of yeast lipidome demonstrated that cantharidin induces substantial changes in the abundance and composition of phospholipids and sphingolipids in CRG1-dependent manner. Finally, we showed that orf19.633, the functional homologue of CRG1 in fungal pathogen Candida albicans, is also involved in lipid homeostasis in response to cantharidin. This work demonstrates the importance of systems-level approach in the characterization of a novel methyltransferase and its role in mediating drug cytotoxicity through lipid homeostasis.

33. iNKT lymphocyte subpopulations in Fabry disease, a glycosphingolipid storage disease.

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Fabry disease is a lysosomal storage disease (LSD) characterized by deficient activity of the alpha galactosidase A enzyme which leads to systemic accumulation of Gb3. For Fabry disease patient's enzymatic replacement therapy (ERT) is available since 2001.

In the Fabry disease mouse model, as well as other LSD mouse models, there is a reduction in the number of invariant Natural Killer T (iNKT) cells. This reduction has been associated with deficient lipid antigen presentation to these cells. A previous study showed that in Fabry disease patients under ERT no alteration was observed in the number of peripheral blood iNKT cells. iNKT cells are a subset of lymphocytes that recognize lipid antigens in the context of CD1d and mediate potent immune regulatory functions via the rapid production of interferon-gama and interleukin-4.

In this meeting we will present the analyses of Fabry disease patients blood iNKT cell subpopulations. Our preliminary results (Fabry patients n=4; control subjects n=5) indicate that there is a clear alteration in the subpopulations of iNKT cells, with a decrease in the percentage of the CD8+, an increase of the CD4-CD8-, and no alteration in the CD4+ iNKT cells.

The physiological function of each iNKT subpopulation is still uncertain however it is known that in the human thymus iNKT cells are mainly CD4+, the CD4-CD8- and CD8+ stages, that are altered in Fabry disease patients, arise post-thymically. This study may contribute both to a better understanding of the mechanisms that regulate the iNKT cells subpopulations and to the understanding of the Fabry disease pathophysiology.

34. The role of membrane lipids from endosomes and lysosomes in the apoptosis induced by *Clostridium perfringens* alpha-toxin

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Clostridium perfringens phospholipase C (Cp-PLC), also called alpha-toxin is considered a prototype of cytotoxic bacterial phospholipases C, and several facts indicate that it is the major virulence factor in C. perfringens-induced gas gangrene. Different investigation results show that alpha-toxin hydrolyses phosphatidylcholine and sphingomyelin at a broad range of pHs, induces platelet aggregation, and is hemolytic, cytotoxic, myotoxic and lethal. Considerable progress has been made during recent years in the knowledge of the mechanism by which alpha-toxin induces platelet aggregation and hemolysis. However, the understanding of the mechanism of toxicity in nucleated cells is incomplete. Although, it's known that at high concentrations alpha-toxin causes membrane disruption and cytolysis. However, at low concentrations, alpha-toxin only causes limited hydrolysis of its substrates leading to the unregulated generation of bioactive lipids and inducing cell death independently of plasma membrane disruption. Moreover, Ganglioside deficient cells, compared with wild type, show an high sensibility to the toxin and undergo apoptosis upon exposure to very low concentrations of a-toxin. The data accumulated support the hypothesis that alpha-toxin undergoes endocytosis via a caveolar-like mechanism and cleaves the lipids of the membranes from the endolysosomal system which somehow triggers apoptosis. Indeed, our unpublished results show that inhibitors of caveolae dependent endocytosis such as Methyl cyclodextrin, filipin and nystatin protect against the apoptotic effect of toxin. The aim of this work will be to characterize the changes of the lipid composition of the membranes from the endolysosomal system in ganglioside deficient cells exposed to alpha-toxin and correlate those changes with the induction of apoptosis. The knowledge obtained would provide new insights towards understanding the mechanism of toxicity of bacterial phospholipases C sim ilar to alpha-toxin and could help to develop novel therapeutic strategies in gas gangrene and other bacterial infections.

35. Association of EDEM's with ERAD substrates and intracellular traffic of these complexes

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Membrane and secretory proteins are synthesized and processed in the ER, and as soon as they enter the ER lumen oligosaccharyl transferase attaches glycans to asparagines residues from the nascent polipeptide. N-linked glycans are recognized by the ER resident molecular chaperones which help the protein to achieve the native conformation. Terminaly misfolded proteins are targeted for degradation by the ERAD (ER-associated degradation).

EDEM (ER degradation-enhancing ?-manosidase like protein) proteins are ERAD components, in the proximity of the export channel, that guide the disposal of misfolded glycoproteins from the ER. EDEM 1 was shown to have a much faster turnover than ER resident chaperones and is segregated from the ER into LC3-I-coated vesicles, the EDEMosomes, and is rapidly degraded by the lysosomal enzymes. Recent studies have shown that endogenous EDEM 1 reaches the cytosol and is degraded by basal autophagy. EDEM 1 was detected by immunocytochemistry in the autophagosomes and biochemically in LC3 immuno-purified autophagosomes.

The main goal of our project is to determine the role of the EDEM proteins in ERAD pathway and the sequence of interactions of ERAD substrates from protein recognition to cytoplasmic degradation. First we have to understand the molecular mechanism that discriminates between native and malfolded proteins, investigate the association of the ERAD substrates with EDEM proteins and establish their role in this pathway.

36. Cholesterol loss during aging in hippocampal neurons. Causes and consequences

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Binding of the neurotrophin BDNF to the TrkB receptor is a major survival mechanism during embryonic development. In the aged brain however, BDNF levels are low, suggesting that additional mechanisms must have developed to activate TrkB and the downstream pro-survival molecules. We show that age-associated increase in TrkB activity is correlated with a mild yet progressive loss of cholesterol. This, in turn, is correlated with increased expression of the cholesterol catabolic enzyme cholesterol 24-hydroxylase. Direct cause-effect, cholesterol loss-high TrkB activity, was demonstrated by pharmacological means and by manipulating the levels of cholesterol 24-hydroxylase. Experiments using farnesy-photoactivatable GFP or single molecule tracking of AMPA receptors indicated that cholesterol loss during aging is also accompanied by plasma membrane rigidization that results in reduced neuronal performance.

37. The effect of N-acyl chain methyl-branching on sterol displacement from sphingomyelin domains and stabilization of bilayers by ceramide

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Long-chain ceramides with saturated *N*-linked acyl chains and sphingosine as the long chain base are known to be effective in increasing the molecular order of bilayer membranes. The ordering effect of these ceramides originates from their structure which enables them to form tightly packed lateral gel-phase domains. A characteristic property of these ceramides is that when present in bilayers that also contain sphingomyelin and cholesterol, the ceramides will compete with cholesterol for interactions with sphingomyelin, resulting in the exclusion of cholesterol from highly ordered domains formed by sphingomyelin and ceramide. It is known from previous studies that modification of the long chain base in or near the polar portion of the head group of ceramide does not alter the properties of the ceramides, but the analogues stabilize bilayers and displace sterol as a normal ceramide (*Megha et al., 2007 Biochim Biophys Acta **1768**, 2205.*). Instead, altering the length of the ceramide *N*-linked acyl chain affected the thermal stability of ceramide-sphingomyelin domains, the domain-melting temperature increasing for ceramides having a *N*-linked acyl chain with 12 or more methylene units and decreasing for shorter chain ceramides (*Nybond et al., 2005 Biochim Biophys Acta **1718**, 61*). The purpose of our study was to introduce methyl-groups to different positions of the *N*-linked acyl chain of ceramide and compare the ability of these branched ceramides to displace cholesterol and affect the order of bilayers. We have synthesized two sets of branched ceramide analogues, one set with sphingosine as the long chain base and the other with phytosphingosine as the long chain base, the methyl-branches being at positions C10 of a 16:0 acyl chain or at the position C15 or C16 of a 17:0 acyl chain. Each set also included a ceramide with phytanic *N*-linked acyl chain, which contains four methyl-groups at positions 3, 7, 11 and 15 of the 16 carbon atoms long chain. In the set of the sphingosine-based analogues, also a hydroxylated (at C2) 18:0 ceramide was included. To better understand which parts of the ceramide molecule contribute to the characteristical behavior of ceramides in bilayer membranes we studied the membrane properties of the branched ceramides by fluorescence anisotropy, lifetime and quenching measurements utilizing mainly diphenylhexatrien, cholestatrienol and *trans*-parinaric acid as the fluorescent reporters. Displacement of sterol by the ceramides was also studied by a newly developed method which measures the partitioning of sterol (cholestatrienol) between a bilayer and cyclodextrin (*Nyholm et al., 2010 Biochim Biophys Acta Epub ahead of print*).

38. Functional characterization of the glycerophosphodiesterase isoform GDE2 in glycerophosphoinositols catabolism

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Phosphatidylinositols undergo sequential actions of phospholipase A2 (PLA2) and lysolipase activities to form lysophosphatidylinositols and glycerophosphoinositols (GPIs), respectively. The glycerophosphodiester phosphodiesterases (GP-PDEs) have recently been involved in the catabolism of GPIs. However, until now there is little information about the complete catabolism and functions of GPIs in different cells. Among the different glycerophosphodiesterase (GDE) isoforms, the substrate specificities and physiological roles have been defined only for GDE1 and GDE3. GDE1 showed preference for the GPIs over glycerophosphocholine as substrate, with glycerophosphoinositol (GroPIns) converted into inositol and glycerol phosphate. While, GDE3 also showed specificity for GroPIns and hydrolyzed it to glycerol and inositol 1-phosphate. Interestingly, overexpression of wild-type GDE3 in osteoblasts promotes disassembly of actin stress fibers, decrease in growth rate, and increase in alka line phosphatase activity and calcium content, indicating a role for GDE3 in induction of differentiation. Here, we looked into the metabolic pathways of the GPIs, specifically looking into the catabolism of GroPIns and GroPIns 4-phosphate by a member of the GDE family: GDE2. We have been investigating the potential catabolic activity and the substrate specificities, by overexpressing GDE2 in HEK293T cells and using these cell lysates as a source of enzyme, while following the reactions by mass spectrometry and HPLC analysis. These in-vitro assays are to be complemented by functional studies using intact GDE2-expressing HEK293T cells and macrophages.

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39. Impairment of cholesterol trafficking plays a role in amyloid beta peptide toxicity

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There is growing evidence linking brain cholesterol to the development of Alzheimer's disease (AD). 1) Epidemiological studies suggested that the use of statins, cholesterol synthesis inhibitors, could reduce the risk of AD, 2) cholesterol levels regulate the production of amyloid beta (Ab) peptide, the major component of the amyloid plaques, and 3) Apolipoprotein E (apoE) isoform-4 is the only known risk factor for AD.

Here, we present a new link between cholesterol and Ab. We discovered that Ab impairs intracellular cholesterol trafficking in neurons. Our hypothesis is that Ab-induced impairment of intracellular cholesterol trafficking contributes to Ab-induced neurotoxicity. Statins could not protect against the impairment of the intracellular cholesterol trafficking observed in Ab-treated neurons. Overexpression of Rab9, a small GTPase that regulate vesicular trafficking from late endosomes to the Golgi, resulted in a significant reduction in Ab-in duced impairment of intracellular cholesterol trafficking and in Ab neuronal toxicity. We studied the effect of Ab on neuronal cholesterol homeostasis and found that it does not cause a change in total neuronal cholesterol mass. Conversely, Ab reduces de novo cholesterol synthesis.

In addition, Ab decreases the anterograde transport of newly synthesized cholesterol from cell bodies to axons, which could play a role in Ab-induced axonal degeneration. This study presents novel and important information including: 1) a mechanistic explanation of the role of cholesterol in Ab neurotoxicity, 2) testing the ability of statins and other therapeutic approaches to protect against Ab toxicity, and 3) studying the intracellular cholesterol trafficking in the nervous system, since most of the intracellular cholesterol trafficking studies were done in non-neuronal systems.

40. The nuclear receptor PPARβ/δ sensitizes skin to UVB-induced carcinoma

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Peroxisome proliferator-activated receptors (PPARs) are ligand-inducible transcription factors identified in the early 1990's as nuclear receptors for compounds causing peroxisome proliferation in rodent liver. Three isotypes, PPARα (NR1C1), PPARβ (NR1C2, also known as PPARδ; called PPARβ/δ) and PPARγ (NR1C3), have been identified in *Xenopus*, fish, mouse and human. PPARs exhibit broad, but isotype-specific tissue expression patterns. Since their discovery, it has become clear that they are critical modulators of environmental/dietary stimuli and are crucial in the regulation of complex pathways of mammalian metabolism. Among PPARs, the functions of PPARβ/δ were only recently identified due, in large part, to recent advances made with null mouse models and the identification of selective ligands.

During the past five years, a number of potential roles for PPARβ/δ in epithelial homeostasis have been described, including the regulation of keratinocyte survival and differentiation by up-regulation notably of the PI3K-dependent signaling pathways. Recent studies have also reported that PPARβ/δ seems to play a positive role in the proliferation of different cell types and that its expression is increased in carcinomas, such as colorectal, head and neck squamous cancers or in human breast cancer cell lines. However, the mode of action of PPARβ/δ in cancer formation remains poorly defined. The purpose of this project is to investigate the molecular mechanism involving PPARβ/δ in UVB-induced skin cancer using hairless/albino mice bearing or not a deletion in *pparβ/δ* as an *in vivo* model.

The results obtained *in vivo* show that *pparβ/δ* is specifically re-expressed upon UVB irradiation, and that wt mice develop papillomas earlier than *pparβ/δ-/-* mice. Analysis of microarray data showed that the expression of the proto-oncogene *c-src* is positively regulated by PPARβ/δ in response to UVB. *In vivo* and using a cell line of human immortalized keratinocytes (HaCat) treated with a PPARb/d-specific agonist, we have demonstrated that this increase in c-Src mRNA expression is correlated with a higher protein expression and kinase activity, reflected in the up-regulation of the EGF-R/Erk1/2 signaling pathway in response to UVB. As c-Src has been described to trigger epithelial to mesenchymal transition (EMT), a mechanism involved in metastasis formation from carcinoma, we have investigated the expression of EMT markers in mice papillomas and have observed that most of them are up-regulated specifically in wt mice compared to null mice. The *c-src* promoter contains several PPAR response elements. Thus, our current work aims to determine whether, on the one hand, *c-src* is a direct PPARβ/δ target gene and, on the other hand, whether PPARβ/δ-induced *c-src* expression is also found in biopsies from skin carcinoma patients. Moreover, we are investigating if the model proposed above occurs in other types of human carcinomas.

Altogether, our results show, for the first time, that PPARβ/δ is involved in the expression of the proto-oncogene *c-src* and reveal new functions for PPARs in cancer. This is of particular interest since specific PPARβ/δ agonists are currently under phase II clinical trials for the treatment of dyslipidemia and may help defining safety guidelines for the use of such drugs.

41. Understanding the secretory pathway: Elucidating the regulation of membrane pumps involved in lipid transport and vesicle biogenesis

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P4-ATPases play a critical role in the biogenesis of transport vesicles in the secretory pathway and their activity is held responsible for creating and maintaining the membrane lipid asymmetry.P4-ATPases form a stable complex with beta-subunits and current evidence strongly suggests that this complex is able to transport phospholipids from the outer to the inner leaflet of biological membranes. In S. cerevisiae two kinases have been found to regulate two plasma membrane P4-ATPases. In this study we will elucidate whether a similar regulation takes place in the plant Arabidopsis thaliana. The study consists of 4 parts: A) Development of a screening system to study regulation of activity, B) Analysis of lipid transport, C) Identification and cloning of plant kinase candidates and D) Verification of protein-protein interaction between kinases and P4-ATPases. A yeast screening system has been developed by knocking out yeast kinases in different combinations with plasma membrane P4-ATPases. Three possible plant kinases have been identified using the TAIR co-expresison database and BLAST similarity searches, and 1 has been cloned.

Preliminary results from the lipid transport assays indeed indicates that the plant P4-ATPase named ALA3 is not functional when expressed in a kinase-deficient yeast strain. By expressing different combinations of P4-ATPases/beta-subunits and kinases in yeast and measuring lipid transport over the plasma membrane we will show if kinase activity is required for phospholipid flipping by plant P4-ATPases. Finally, to verify in vivo protein-protein interaction between kinases and the P4-ATPase/beta-subunit complex, the split-ubiquitin system will be used.

42. Ablation of the very long chain fatty acid elongase ELOVL3 in mice leads to constrained lipid storage and resistance to diet-induced obesity

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Although saturated and monounsaturated very-long-chain fatty acids (VLCFA) have long been associated with undesirable effects on health, including obesity, heart failure and atherosclerosis, the physiological role of endogenous synthesis is largely unknown. The fatty acid elongase ELOVL3 is controlling the synthesis of C20-C24 saturated and monounsaturated VLCFA mainly in liver, brown and white adipose tissue and in triglyceride rich glands such as the sebaceous and meibomian glands. Here we show that ablation of ELOVL3 leads to reduced adiponectin levels, constrained expansion of adipose tissue and resistance against diet-induced obesity, a situation that is more exaggerated in female mice. Both female and male knockout mice show reduced hepatic lipogenic gene expression and triglyceride content, a situation, which is associated with, reduced expression of PPARg and its target genes.

43. Changes in Sphingomyelins and Ceramides with very Long-Chain Polyunsaturated Fatty Acids During Spermatogenic Cell Differentiation

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In seminiferous tubules, Sertoli cells and spermatogenic cells at different stages of differentiation coexist. In this study, pachytene spermatocytes and round spermatids were isolated from rat seminiferous tubules to study how ceramides (Cer) and sphingomyelins (SM) with very long chain polyunsaturated fatty acids (VLCPUFA) change during spermatogenesis. Although the concentration of phospholipids including SM was similar in both cell types, there were many differences in their fatty acids (FA). SM and Cer had 28:4n-6, 30:5n-6 and 32:5n-6 as their main FA in spermatocytes, but both lipids contained mostly 28:4n-6 in spermatids. In turn, spermatids, but not spermatocytes, contained an important proportion of 2-hydroxylated versions of the mentioned VLCPUFA (2-OH VLCPUFA). The 2-OH VLCPUFA / non-hydroxy VLCPUFA ratio not only increased markedly in SM and Cer with germ cell differentiation but was even higher in (epididymal) spermatozoa. Although the enzyme that is responsible for the synthesis of the 2-OH VLCPUFA of SM and Cer remains to be investigated, our results suggest that it is expressed after completion of meiosis. Residual bodies, which contain materials -including lipids- that are discarded from late spermatids (elongating-condensing forms) as they differentiate to spermatozoa, were unusual in that they contained no Cer but plenty of SM with almost exclusively 2-OH VLCPUFA. Taking into account that residual bodies are normally phagocytosed by Sertoli cells, the eventual fate of these SM species is another intriguing question to be solved regarding sphingolipid metabolism during the spermatogenic cycle.

44. Effect of sterol structure on membrane domain formation in sphingomyelin-sterol liposomes as studied by EPR

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Ostreolysin is a cytolytic protein from the edible oyster mushroom (Pleurotus ostreatus), which recognizes specifically and binds to raft-like sterol-enriched membrane domains that exist in the liquid-ordered phase. Its binding can be abolished by micromolar concentrations of lysophospholipids and fatty acids. The membrane activity of ostreolysin, however, does not completely correlate with the ability of a certain sterol to induce the formation of a liquid-ordered phase, suggesting that the protein requires an additional structural organization of the membrane to exert its activity. The aim of this study was to further characterize the lipid membranes that facilitate ostreolysin binding by analyzing their lipid phase domain structure. Electron paramagnetic resonance (EPR) was used to analyze the ordering and dynamics of membrane lipids and the membrane domain structure of a series of unilamellar liposomes prepared by systematically changing the lipid components. Our results corroborate the earlier conclusion that the average membrane fluidity of ostreolysin-susceptible liposomes alone cannot account for the membrane activity of the protein. Combined with previous data computer-aided interpretation of EPR spectra strongly suggests that chemical properties of membrane constituents, their specific distribution, and physical characteristics of membrane nanodomains, resulting from the presence of sterol and sphingomyelin (or a highly ordered phospholipid, dipalmitoylphosphatidylcholine), are essential prerequisites for ostreolysin membrane binding and pore-formation.

45. The role of phosphorylation in the regulation of adipose triglyceride lipase activity

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Lipolysis, i. e. the breakdown of triglyceride (TG) stores, is catalysed by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL) in consecutive steps. This process underlies hormonal regulation. Activation of lipolysis involves phosphorylation of several proteins (including HSL), altering their activities and/or intracellular localisations. ATGL, the rate-limiting enzyme of lipolysis, has been shown to be phosphorylated at two sites (serines 406 and 430). However, the regulatory role of these phosphorylation sites has not been investigated so far in detail.

Here, we investigated whether phosphorylation of ATGL affects the enzymatic activity of the enzyme in vitro and in living cells. Dephosphorylation of ATGL by lambda-phosphatase or mutation of the two known phosphorylation sites to alanines (S406A and S430A) did not alter TG hydrolase activity in vitro using an artificial substrate containing radiolabeled triolein. However, in living cells the ATGL mutant S406A was less efficient in mobilizing TG as compared to the wild-type enzyme.

This defect could be partly explained by reduced lipid droplet localisation of S406A. These observations suggest that protein phosphorylation affects rather the cellular localisation of ATGL than its in vitro activity. Interestingly, we found that mutation of the two reported phosphorylation sites did not lead to complete loss of ATGL phosphorylation suggesting that at least one additional phosphorylation site exists which is possibly involved in the regulation of enzyme activity.

Together, results of this study indicate that, similar as reported for HSL, also the cellular localisation of ATGL is regulated by phosphorylation events. Further studies are required to identify all phosphorylation sites of the enzyme and the kinases mediating ATGL phosphoylation.

46. #Genome-wide Unsaturated Fatty Acid Sensitivity Screen in Yeast Identifies Novel Pathways in Lipid Droplet Metabolism

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Under conditions of excess lipid exposure, a cells first line of defense against lipid accumulation is the esterification of fatty acids into TG and their subsequent storage in the lipid droplet. Defects in lipid droplet formation compromise the cells ability to store neutral lipids and increases their sensitivity to fatty acid exposure and lipotoxicity. Abnormal accumulation of lipid droplets in skeletal muscle, hepatocytes and pancreatic β cells is associated with the development of type 2 diabetes, heart disease and steatohepatitis. Understanding the details of lipid droplet formation, fusion and metabolism are therefore important aspects in prevention of these diseases. Our laboratory has found that neutral lipid deficient yeast strains are unable to grow in the presence of unsaturated fatty acids (UFA), such as palmitoleate (PO). From this we hypothesized that sensitivity of other strains to PO is an indicator of lipid droplet status. A genome-wide PO sensitivity s creen of single mutants was completed in order to identify novel candidate genes involved in lipid droplet metabolism. From this screen, we have identified several gene knockout mutants with robust UFA sensitivity phenotypes. Interestingly, several of the identified genes are involved in vesicular protein sorting, sphingolipid metabolism and ER protein insertion and we have chosen to focus on these protein families for further investigation. We have identified lipid droplet phenotypes and aberrations in triglyceride and sterol ester accumulation in many of these mutants that are likely associated with their fatty acid sensitivity and hypothesize that these proteins play novel roles in lipid droplet homeostasis.

47. #An unconventional biosynthetic route for Cardiolipin in Trypanosoma brucei

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The phospholipid composition defines the physical properties of a biological membrane, such as curvature and permeability, and affects the assembly and function of protein complexes in a given membrane. Mitochondria contain highly organized membrane structures, and their formation and homeostasis is of critical importance for cell survival.

The protozoan parasite, Trypanosoma brucei, is the causative agent of human African sleeping sickness and a related animal disease, nagana. During their life cycle, trypanosomes alternate between two vastly different host environments, the mammalian bloodstream and several compartments of the insect (tsetse) vector. Since T. brucei parasites contain a single mitochondrion, which fulfils completely different functions during the two major life cycle stages, it has become an interesting model organism to study mitochondrial biogenesis and fission, protein and tRNA import into and metabolic function of mitochondria.

Our laboratory has previously shown that down-regulation of phosphatidylethanolamine (PE) synthesis in T. brucei causes abnormal cristae morphology and fragmented mitochondria (Signorell et al., Mol Microbiol. 2009;72:1068). We now extend these studies by examining the role of the mitochondria-specific glycerophospholipid, cardiolipin (CL), in mitochondrial homeostasis. In T. brucei, CL synthesis is proposed to occur via the consecutive action of a newly identified phosphatidylglycerophosphate (PGP) synthase, a yet unknown PGP phosphatase, and a newly identified prokaryotic-type CL synthase. Using GFP-tagged constructs, we found that both PGP synthase and CL synthase localize to mitochondria. In addition, gene knock-down of PGP synthase by RNAi had a strong effect on cell growth in procyclic form T. brucei and caused a decrease in PG levels, but didn't affect CL levels after 3 days of RNAi induction. In contrast, RNAi against CL synthase had only little effect on cell grow th. CL levels decreased to about 50% after 6 days of RNAi induction, concomitant with an increase in PG levels to about 150%. To our knowledge, this is the first time that a prokaryotic-type CL synthase is identified in a eukaryotic organism. Future work will focus on mitochondrial defects caused by depletion of PG and/or CL and on the biochemical characterization of the T. brucei CL synthase.

48. The Structure of Human Tear Fluid at The Air-Water Interface - a Computational Study

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The tear fluid is a water solution rich in lipids (fat molecules). It disinfects the surface of the eye and protects it from drying. The lipids of the tear fluid form a monolayer between the air and the water solution, preventing the vaporization of water molecules from the surface of the eye. If the lipid composition of tear fluid is abnormal, for example due to long days in front of computer, so called "dry eyes" can result. "Dry eyes" can be treated with liquid mixtures which balance the lipid composition of the eye, but those products are often not very effective. To improve the effectivity of these mixtures the properties of the lipid layer between the eye surface and air needs to be investigated.

There are only a few computational models of lipid layers in air-water interface [1]. Here we present a simulation of the lipid layer of tear fluid, constructed from experimental lipid composition and modelled with Martini coarse grained force field, designed especially for simulations of lipids and other biomolecules.
The simulations with Gromacs software allow us to elucidate structural and dynamical properties of the tear fluid systems, and in particular the compression isotherms, that describe the equation of state for these complex lipid systems. The results will be discussed in detail. This project is being carried out together with our experimental collaborators (J. Holopainen et al.)

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49. Polymorphisms of apolipoprotien(a) isoforms and lipoprotein(a) in a Macedonian population

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High plasma levels of Lipoprotein(a) [Lp(a)] increase the risk of premature atherosclerosis. Lp(a) contains a unique protein, apolipoprotein(a) [apo(a)]. Apo(a) shows a high degree of genetic polymorphisms, resulting from variable number of tandem repeats of K-IV type 2 in the LPA gene. 180 healthy blood donors, aged between 18 and 60 years (94 males, 86 fe¬males), and 100 healthy children aged between 9 and 18 years (51 boys and 49 girls) were included in the study. Denaturing 3-15% gradient SDS-PAGE was used for the apo(a) isoforms separation.

The results showed that the frequency distribution of apo(a) isoforms (B, S1, S3, S4, >S4) fit the expectation of the Hardy-Weinberg equilibrium. The frequencies of the six alleles were: B= 0.022, S1 = 0.028, S3= 0.201, S4= 0.397, >S4=0.110, and 0 = 0.242. The distribution of alleles was skewed towards alleles encoding large apo(a) isoforms associated with low Lp(a) levels. Large apo(a) isoforms (S4 and S3 from single banded and S4S3 from double-banded) were most prevalent in children and in adults. 164 of the adults (91.1%) and 98% of the children had a Lp(a) level beneath the 30 mg/dL, with 77% showing levels of 10 mg/dL or less. We found a significant inverse correlation ((r) = –0.3477, $p < 0.001$) between the Mr of apo(a) isoforms and plasma levels of Lp(a) in adults and in children ((r) = –0.4257, $p < 0.001$).

Our results indicate that apo(a) isoforms are primarily genetically-determined, and are not affected by the plasma lipid and apolipoprotein concentration.

50. Ceramide transfer protein (CERT) expression modulates EGF receptor signalling

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Lipids, as the predominant constituents of cellular membranes, are essential cellular components assuring the structural and functional integrity of cells. The lipid composition of the plasma membrane determines its fluidity and is the basis for the formation of membrane microdomains. These so-called lipid rafts are enriched in cholesterol and sphingomyelin whose synthesis primarily occurs at the Golgi complex. The lipid transfer protein CERT has emerged as a crucial protein for the non-vesicular shuttling of ceramide from the ER to the Golgi where ceramide is converted to sphingomyelin. Little is known about the mechanisms by which local lipid metabolism at the Golgi complex affects signalling events at the plasma membrane. We are able to show that CERT depletion positively affects ligand-induced EGFR signalling and internalization. Similar results were obtained by downregulation of sphingomyelin synthases, suggesting that plasma membrane sphingomyelin levels directly impact on EGFR signalling competence.

51. Regulation of cardiolipin and phosphatidylethanolamine in mitochondria by conserved proteins in the mitochondrial intermembrane space

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Mitochondria are double-membrane enclosed organelles, which harbor diverse biochemical pathways and are essential for eukaryotic cells. Mitochondrial membranes contain the diphosphatidylglycerol lipid cardiolipin (CL), which has pleiotropic roles for mitochondrial activities including the assembly of the respiratory chain, the stability of mitochondrial DNA, protein import and apoptotic processes. Impairment of CL biosynthesis is associated with cardiomyopathy in Barth syndrome. A recent genetic screen identified Ups1 and Ups2/Gep1 as conserved regulators in the mitochondrial intermembrane space, which coordinately control the levels of cardiolipin and the related phospholipid phosphatidylethanolamine. In order to elucidate the function of Ups1 and Ups2/Gep1 in the control of mitochondrial phospholipid levels we expressed tagged versions and identified novel protein interactions. Further functional analysis points to a network of conserved proteins in the intermembrane space of mitochondria required to ensure proper phospholipid composition of mitochondrial and cellular membranes.

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52. The role of glycosphingolipid and heparan sulfate in promoting membrane pore formation and internalization of basic proteins from cobra venom

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Cobra venom contains basic proteins of homologue cardiotoxins (CTXs) that induce tissue necrosis and systolic heart arrest in bitten victims. CTX-induced membrane pore formation is one of the major mechanisms responsible for the venom's designated cytotoxicity. We examine how glycoconjugates such as heparan sulfates (HS) and glycosphingolipids, located respectively in the extracellular matrix and lipid bilayers of the cell membranes, facilitate CTX pore formation and internalization. Evidences for HS-facilitated cell surface retention and glycosphingolipid-facilitated membrane bilayer insertion of CTX are presented to suggest that similar physical steps could play a role in the mediation of other pore forming toxins (PFT). The membrane pores formed by PFT are expected to have limited lifetime on biological cell surface as a result of membrane dynamics during endocytosis and/or rearrangement of lipid rafts. The endocytic pathway of CTX homologues are also shown to be highly sensitive to the cholesterol and calcium content of the studied cells and its binding specificity toward HS and glycosphingolipid in the membrane surface, suggesting a role of glycoconjugates in modulating membrane domains involving in endocytic pathway.

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53. ADF/cofilin binds phosphoinositides in a multivalent manner to act as a PIP2-density sensor

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Actin-depolymerizing-factor (ADF)/cofilins have emerged as key regulators of cytoskeletal dynamics in cell motility, morphogenesis, endocytosis and cytokinesis. The activities of ADF/cofilins are regulated by membrane phospholipid PI(4,5)P2 in vitro and in cells, but the mechanism of ADF/cofilin - PI(4,5)P2 interaction has remained controversial. Interestingly, recent studies suggested that ADF/cofilins interact with PI(4,5)P2 through a specific binding pocket and that this interaction is dependent on pH. Here, we combined systematic mutagenesis with biochemical and spectroscopic methods to elucidate the phosphoinositide-binding mechanism of ADF/cofilins. Our analysis revealed that cofilin does not harbor a specific PI(4,5)P2-binding pocket, but instead interacts with PI(4,5)P2 through a large positively charged surface of the molecule. Importantly, cofilin interacts simultaneously with multiple PI(4,5)P2 headgroups in a cooperative manner. Consequently, interactions of cofil in with membranes and actin exhibit sharp sensitivity to PI(4,5)P2-density. Finally, we show that cofilin binding to PI(4,5)P2 is not sensitive to changes in the pH at physiological salt concentration, although the PI(4,5)P2-clustering activity of cofilin is moderately inhibited at elevated pH. Collectively, our data demonstrating that ADF/cofilins bind PI(4,5)P2 headgroups through multivalent, cooperative mechanism, suggest that the actin filament disassembly activity of ADF/cofilin can be accurately regulated by small changes in the PI(4,5)P2-density at cellular membranes.