THE ISLAND OF SPETSAI

The island of Spetsai is situated at the entrance of the Argolikos bay, and its area is 22.24 square kilometers.

Pausanias, the Greek traveler and historian of the 2nd century A.D., whose work Hellados Periegesis (Travelling through Greece) is an invaluable source of information for archaeologists today, calls the island Pityousa (from the word pitys that means pine) because the place was full of pine trees. Later on, the name changed to Petsa and afterwards to Spetsai (plural) [Ed. note: Contemporary common usage is Spetses].

Although inhabited from ancient times, as it is testified from the findings of ancient tombs and coins, its population changed many times because the island, very often, was invaded by the pirates of the Aegean sea, forcing thus the islanders to flee to safer places in the mainland. When things became quiet again, other Greeks from the Peloponnesus or other islands would come to settle to Spetsai, till the next wave of pirates would force them to leave the island again.

From the times of the Crusades and till the beginning of the 18th century, when they became part of the Ottoman Empire, the Aegean Islands were under Venetian or Genoese rule. Spetsai were seized by the Turks in 1715, but very soon, as it happened with most of the islands, they were given considerable privileges which eventually amounted to autonomy; that meant that by paying taxes to the conqueror, the islanders were left alone to busy themselves with commerce and with building of ships. From the middle of the 18th century we have the emergence of a Greek merchant fleet. Spetsai, along with two other Greek islands, the nearly Hydra and another small island close to the Asiatic coast, Psara, were destined to develop the largest merchant fleet in the last quarter of the 18th century.

From this time, the Spetsiotes (the inhabitants of Spetsai) became very active as merchants with business interests in Europe, Africa, and Asia, accumulating considerable wealth, so that they were counted among the richest of Greeks. F.C.H.L. Pouqueville, the French philhellene, in his Voyage de la Grece, published in Paris in 1826-1827 (VI, 294-297) says that around 1813 the island of Spetsai had 60 ships, whose capacity was 19,500 tons, with a total crew of 2,700 seamen and 900 canons.

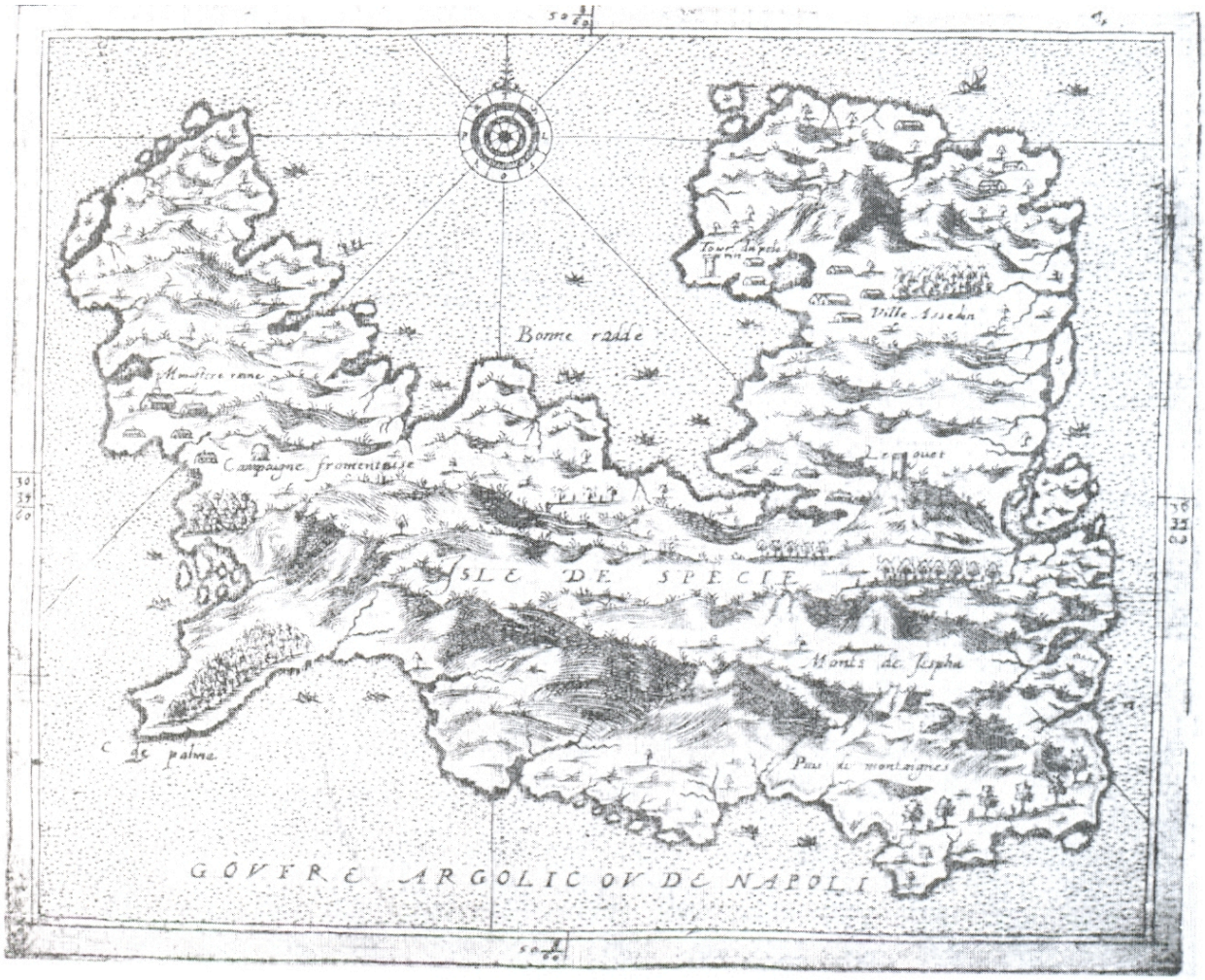
After the outbreak of the Greek War of Independence in 1821, the Greek merchant marine was converted into a revolutionary naval force. The three major maritime islands, Hydra, Spetsai, and Psara, played a decisive role in guarding the coasts against Turkish invasions and controlling the Aegean sea.

Among the figures distinguished for their courage and accomplishments" during the War, a unique personality is the heroine Laskarina Bouboulina (1771-1825), called by a contemporary Englishman who personally met her "A true descendant of the women of Sparta". Widow of a rich Spetsiot, she not only offered all her wealth and small fleet of ships for the Greek cause, but also she took part herself in the naval encounters with the Ottoman fleets. Her personality has become a legend and she is the subject of many tales and poems. Her relics are at the Museum of Spetsai and her statue adorns the island. Another important figure in the history of Spetsai is Hatziyannis-Mexis (1751- 1844) whose house, built in 1795-1798 in arabian-venitian style, houses today the Museum of Spetsai.

After a short period of decline because of the War of Independence, the merchant marine was able to recover rapidly but the beginning of the end was close. By 1850, the introduction of steam navigation had a negative effect on the growth of the Greek merchant marine, until the end of the 19th century when the new era for the Greek maritime commerce begins.

Today the island of Spetsai is no longer an important maritime center. It has about 3,500 inhabitants and it is a popular summer resort favored not only by Greeks but by many tourists as well.

Sophie Papageorgiou, Gennadius Library,
Athens, 1984



Eukaryotic Lipids; treasure of regulatory information

FEBS/IUBMB Workshop

Spetses, Greece, June 19 – 24, 2010

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09:00 - 09:50	**Gerrit van Meer** (Utrecht, Netherlands) Simple lipids and cellular physiology
09:50 - 10:40	**Banafshe Larijani** (London, UK) Proteo-lipid regulation in nuclear envelope assembly
10:40 - 11:00	*Coffee break*
11:00 - 11:50	**Daniela Corda** (Naples, Italy) Glycerophosphoinositols and the actin cytoskeleton
11:50 - 12:10	**Shishir Jaikishan** (Turku, Finland) Sphingomyelin analogues with differently branched N-acyl chains: effect on acyl chain order and sterol interaction in bilayer membranes
12:10 - 12:30	**Andrea Balreira** (Porto, Portugal) LIMP-2 sorting receptor of β-glucocerebrosidase: a cell-type specific mechanism
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Monday, June 21

Morning Chair:	***Gennaro de Libero***
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Session 2:	***Lipid signaling***
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11:50 - 12:10	**Michael Bohdanowicz** (Toronto, Canada) PI(3)P-dependent polymerization of actin propels
12:10 - 12:30	**Martin Kreim** (Graz, Austria) Identification and investigation of novel proteins involved in lipid droplet morphology and protein targeting in yeast
12:30 - 12:50	**Ildiko Kasza** (Budapest, Hungary) Effect of cholesterol-lowering drugs on human ABCA1 cell surface expression
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Morning Chair:	***Miriam Greenberg***
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10:40 - 11:00	*Coffee break*
11:00 - 11:50	**Symeon Siniossoglou** (Cambridge, UK) The role of PA metabolism in nuclear membrane
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Wednesday, June 23
Morning Chair: ***Daniela Corda***

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09:50 - 10:40	**Ron Schnaar** (Baltimore, USA) Gangliosides in cell-cell recognition and signaling
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11:00 - 11:50	**Gennaro de Libero** (Basel, Switzerland) Glycolipids and immunotherapy
11:50 - 12:10	**Mauro Serricchio** (Bern, Switzerland) An unconventional biosynthetic route for Cardiolipin in Trypanosoma brucei
12:10 - 12:30	**Kelly Ruggles** (New York, USA) Genome-wide unsaturated fatty acid sensitivity screen in yeast identifies novel pathways in lipid droplet metabolism
12:30 - 12:50	**Hye Won Kang** (Boston, USA) Linking lipid binding to glucose metabolism: increased insulin sensitivity in mice with homozygous disruption of thioesterase superfamily member (Them) 2
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Thursday, June 24 *departure*

Speaker's abstracts in order of the program

The name of the lecturer is underlined and only her/his lab is mentioned.

Sunday, June 20, 9 a.m.

Simple lipids and cellular physiology

Gerrit van Meer

Membrane Enzymology, Bijvoet Centre and Institute of Biomembranes, Utrecht University, Utrecht, The Netherlands.

When acquiring internal membranes and vesicular transport, eukaryotic cells started to synthesize sphingolipids and sterols. The physical differences between these and the glycerophospholipids must have enabled the cells to segregate lipids in the membrane plane. Localizing this event to the Golgi then allowed them to create membranes of different lipid composition, notably a thin, flexible ER membrane, consisting of glycerolipids, and a sturdy plasma membrane containing at least 50% sphingolipids and sterols. Besides sorting membrane proteins, in the course of evolution the simple sphingolipids obtained key positions in cellular physiology by developing specific interactions with (membrane) proteins involved in the execution and control of signaling. The few signaling sphingolipids in mammals must provide basic transmission principles that evolution has built upon for organizing the specific regulatory pathways tuned to the needs of the different cell types in the body.

The phosphosphingolipids sphingomyelin in mammals and inositolphosphoceramide, in yeast and plants, are synthesized on the non-cytosolic surface of the Golgi, just like the glycosphingolipids having two or more sugars. In contrast, glucosylceramide is synthesized on the cytosolic surface of the Golgi in mammals and of the ER in flies and plants. Galactosylceramide is synthesized on the lumenal surface of the ER of specialized mammalian cells. It is a challenge to map the transport pathways of these monoglycosylsphingolipids through the cytosol, across membranes, and along vesicular transport pathways, and to identify the proteins involved. It is an even bigger challenge to find out how cells have learned to use these molecules and their behavior to regulate membrane protein dynamics and function. This must be seen against the background of the general cellular machineries that move lipids across and between membranes. Evidence will be presented that the monoglycosylceramides are key molecules in regulating the activity of the vacuolar proton pump.

Sunday, June 20, 9.50 a.m.

Proteo-lipid regulation in nuclear envelope assembly

Banafshe Larijani and Dominic Poccia

Cell Biophysics, Lincoln's Inn Fields Laboratories, Cancer Research UK, London, U.K.

Regulation of nuclear envelope dynamics is an important example of the universal phenomena of membrane fusion and fission. The nuclear envelope is disassembled and reassembled at each mitosis in typical animal cells. It is not a passive membrane in that its reformation is central to proper cell functioning. To understand the complex architecture of the nuclear envelope is important, as its correct formation is a requirement for all cells that breakdown their nuclear envelope at mitosis. The dysfunction of nuclear envelope assembly leads to different forms of human disease from various types of cancer to Emery-Dreifus muscular dystrophy. In the past years there has been much debate on the mechanism of nuclear envelope assembly, and to date this complex issue has not been fully addressed. In contrast to the typical focus on the structural proteins we have focused our investigations on the role of lipids and their modifying enzymes in nuclear envelope assembly. In conjunction, we have used nuclear envelope formation as a model for characterising the molecular dynamics of membrane fusion. We have shown the involvement of lipids in the regulation of nuclear envelope assembly. A non-endoplasmic reticulum membrane fraction enriched in PLCγ both in vivo and in vitro has elevated amounts of phosphoinositides. Its involvement is critical for nuclear envelope formation and perhaps for other localised membrane fusion events. The discovery of this non-endoplasmic reticulum membrane fraction with atypical levels of phosphoinositides (60mol%) has led to the hypothesis that this family of lipids are not only transient signals but they affect membrane dynamics rendering them both the property of signalling and membrane modifying molecules. To provide evidence for this hypothesis we have developed solid-state nuclear magnetic resonance spectroscopy methods, to define the structure and dynamics of the natural membrane domains in our model.

Our future work in the regulation of nuclear envelope assembly encompasses the role of lipids and their modifying enzymes in both somatic and non-somatic cells.

Nano-analytical tools such as fluorescence lifetime imaging microscopy (FLIM), NMR spectroscopy and liquid chromatography tandem mass spectrometry (LC-MS/MS) are used to provide increased insight into molecular composition and associations during nuclear envelope assembly.

Sunday, June 20, 11 a.m.

The glycerophosphoinositols in the macrophage inflammatory response

Stefania Mariggiò, Pasquale Zizza, Alessia Varone, Virgilio Evangelista and Daniela Corda

Institute of Protein Biochemistry, National Research Council, Naples, Italy

The glycerophosphoinositols (GPIs) are produced in cells through phospholipase A_2 and lysolipase actions on membrane phosphoinositides, and their intracellular concentrations vary according to oncogenic transformation, cell differentiation and hormonal stimulation (1, 2). The rate of GPI synthesis in different cell types is proportional to phospholipase A_2 activity of the producing cells, which varies greatly across cell types. The catabolism of the GPIs is via specific glycerophosphodiester phosphodiesterase actions that are also under hormonal regulation. The main intracellular signalling pathways upon which the GPIs have modulatory roles include adenylyl cyclase, intracellular Ca^{2+} levels, and Rho-GTPase signalling, and they can have diverse effects across multiple cell types. These include modulation of the actin cytoskeleton in fibroblasts, induction of cell proliferation in thyroid cells, and reduction of invasive potential of tumour cell lines. With high phospholipase A_2 activity in some haematopoietic cell lines, a role for the GPIs during inflammation has also been suggested. To define the mechanisms of action of the GPIs via their interacting proteins, we have used biotinylated GPIs (biotin moiety bound to their glycerol backbone) in a proteomic approach based on high-throughput differential LC-MS/MS analysis. Considering GPIs-mediated modulation of the actin cytoskeleton, among the targets identified to date we have focussed on Src-homology-phosphatase-1 (Shp-1), a well-known regulator of Src activation, and shown that it is a direct target of the GPIs.

We are also focussing on the effects of the GPIs in inflammatory and immune responses based on their enhancement of cytokine-dependent chemotaxis in T lymphocytes and modulation of proliferation of lymphocytes induced by T-cell receptor activation. These data indicate roles for the GPIs as modulators of T-cell signalling and T-cell responses. Immune cells also have a potent and regulated phospholipase A_2 activity that provides fine modulation of intracellular GPIs levels consequent to cell development, differentiation and hormone stimulation (i.e. exposure to lipopolysaccharides [LPS], cytokines, and other pro-inflammatory agents). In LPS-stimulated human monocytes, the GPIs are part of a negative feedback loop that limits pro-inflammatory and pro-thrombotic responses, and the pro-coagulant activity here is mainly ascribed to tissue-factor expression. Indeed, LPS induce an increase in mRNA levels of tissue factor, cyclooxygenase-2, interleukin-1beta, and tumour necrosis factor-alpha. Pre-treatment of monocytes with GPIs before LPS stimulation resulted in dose-dependent inhibition of tissue-factor activity, as well as decreases in mRNA levels of all analysed inflammatory genes. Notably, treatment with GPIs was consistently associated with decreased LPS-induced nuclear translocation of transcription factors, such as NFkB. These data support the conclusion that the GPIs have roles as endogenous anti-inflammatory mediators for inflammation resolution.

1. Corda D, Iurisci C, Berrie CP. (2002) Biological activities and metabolism of the lysophosphoinositides and glycerophosphoinositols. Biochim Biophys Acta. 1582:52-69.
2. Corda D, Zizza P, Varone A, Filippi B M, Mariggiò S. (2009) The glycerophosphoinositols: cellular metabolism and biological functions. Cell Mol Life Sci. 66:3449-67.

Sunday, June 20, 11.50 a.m.

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

Shishir Jaikishan, Anders Björkbom and J.Peter Slotte

Department of Biosciences, Biocity, Åbo Akademi University, Turku, Finland,

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.

Sunday, June 20, 12.10 a.m.

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

Andrea Balreira, Paulo Gaspar, Daniel Caiola, João Chaves, Idalina Beirão, José Lopes Lima, Maria do Carmo Macário, Anabela Matos, Jorge Eduardo Azevedo, and Maria Clara Sá Miranda

Unidade de Biologia do Lisossoma e do Peroxissoma (UNILIPE), Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal.

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.

Sunday, June 20, 12.30 a.m.

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

Maria Virginia Borroni and F. J. Barrantes

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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.

Sunday, June 20, 5.30 p.m.

The ABCs of sterol transport

Peter E. Edwards

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ABCG1 has been shown to increase the efflux of cellular sterols to a variety of exogenous acceptors that include HDL and lipid liposomes. In contrast, ABCA1 effluxes both cellular cholesterol and phospholipids to exogenous lipid-poor apoA1. In attempts to identify the mechanisms involved in such sterol efflux we have used biotinylation of endogenous surface proteins and immuno-localization to demonstrate that ABCG1 localizes to vesicles in the endosomal pathway but is undetectable at the cell surface. As expected, ABCA1 is present at the cell surface.

This and other data suggest that the primary function of ABCG1 is to control intracellular sterol movement. In addition, we have identified specific oxysterols that accumulate in macrophages and tissues of Abcg1-/-Apoe-/- DKO mice. These same oxysterols promote apoptosis and likely account for the increase in apoptotic cells noted in atherosclerotic lesions of the hyperlipidemic DKO mice.

Monday, June 21, 9 a.m.

Flexible information processing through lipid signalling: lessons from a billion years of sterol regulation

Randy Hampton

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Sterol molecules are amongst the oldest biomolecules on the planet. Thus, all eukaryotes and many microorganisms share the key features of the mevalonate pathway that produces sterols such as cholesterol and a plethora of other isoprenoids. This high conservation of the sterol pathway between highly diverged organisms presents unique opportunities in the study of regulatory evolution and adaptability. HMG-CoA reductase (HMGR) is one of the early committed steps in sterol synthesis, and in many circumstances it is the rate-determining step for sterol synthesis. Accordingly, HMGR undergoes feedback regulation of its stability as part of the multivalent control of the sterol pathway that allows cells to vary flux as cellular demand for isoprenoids varies. When mevalonate pathway activity is high, degradation of HMGR is high and the levels tend to be kept low. Conversely, when pathway activity is slowed, HMGR stability increases and the enzyme levels increase. Because regulation of HMGR stability is conserved between S. cerevisiae and humans, we capitalized on the genetic facility of yeast to discover the mechanism of HMGR stability control. Not surprisingly, the basic molecular features of regulated degradation of HMGR are also conserved between yeast and mammals. However, there are some clear differences between the two organisms that provide a unique vantage point to examine evolutionary aspects of regulation. In this talk the two systems will be contrasted and compared. We will describe our recent studies showing how the same conserved proteins, motifs and mechanism are used to effect distinct outcomes appropriate for the differing roles of sterols and HMGR in the yeast and the mammal. Furthermore, we will speculate about unknown aspects of sterol pathway regulation in both yeast and mammals, using information gleaned from each organism to suggest reasonable hypotheses for the nature of those unknowns in the other.

Monday, June 21, 9.50 a.m.

PC-TP/StarD2: Linking membranes to metabolism

David Cohen

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Phosphatidylcholine transfer protein (PC-TP) binds phosphatidylcholines and catalyzes their intermembrane transfer and exchange in vitro. PC-TP (synonym StARD2) is a member of the steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain superfamily, with a structure that comprises a hydrophobic pocket and a well-defined head-group binding site. Recent studies have revealed key regulatory roles for PC-TP in lipid and glucose metabolism. Notably, Pctp-/- mice are sensitized to insulin action and exhibit more efficient brown fat-mediated thermogenesis. PC-TP appears to limit access of fatty acids to mitochondria, potentially by stimulating the activity of thioesterase superfamily member 2 (Them2), a newly characterized long-chain fatty acyl-CoA thioesterase. Regulation of PC-TP expression by peroxisome proliferator activated receptor α (PPARα) further supports its role in nutrient homeostasis. Because PC-TP discriminates among phosphatidylcholi nes within lipid bilayers, it may function as a sensor that links metabolic regulation to membrane composition.

Monday, June 21, 11 a.m.

Phosphoinositide in signaling and disease

Bernard Payrastre

Inserm U563, Département d'Oncogenèse, signalisation et innovation thérapeutique and Université Toulouse III Paul Sabatier, Toulouse, France.

Phosphoinositides (PIs) play important roles in the control of several cell functions including survival, growth or motility. Initially, phosphoinositide signaling was thought to work exclusively through the PLC pathway, but it is now clear that the seven polyphosphoinositides can function as lipid messengers. They can interact with protein modules such as PH, FYVE, PX, PHD or ENTH domains allowing recruitment and activation of cellular effectors involved in specific signaling. Therefore, not only PI 3-kinase products, but also most of the other PIs are important spatio-temporal organizers of key intracellular regulatory pathways. Controlling the level and the compartmentalization of phosphoinositides is therefore critical and several specific phosphoinositide -kinases and -phosphatases have been directly implicated in human diseases. After a general introduction on the metabolism and functions of these lipids, we will present and discuss our results concerning one the most recently discovered phosphoinositide, phosphatidylinositol 5-monophosphate (PtdIns5P).

Interestingly, during the first steps of Shigella flexneri infection, the bacterial phosphatase IpgD is injected into the host cell where it specifically transforms PtdIns(4,5)P2 into PtdIns5P, which has important consequences for the host cell fate. Besides morphological effects and cytoskeleton reorganization, IpgD activates the PI 3-kinase/Akt survival pathway via PtdIns5P production. We will describe how PtdIns5P can hijack a growth factor signaling and prevent its termination.

Monday, June 21, 11.50 a.m.

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

Michal Bohdanowicz, G. Cosio, J. Bakker, S. Grinstein

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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.

Monday, June 21, 12.10 a.m.

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

Kreim, M., Natter, K., Kohlwein, S.D.

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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.

Monday, June 21, 12.30 a.m.

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.

Monday, June 21, 5.30 p.m.

PPARs stand at the crossroads of metabolism, inflammation and cancer

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Peroxisome proliferator-activated receptors form a small family of three nuclear receptors (PPARalpha, PPARbeta, also called PPARdelta and PPARgamma), which act as lipid sensors to modulate gene expression.

PPARgamma plays a key role in adipocyte differentiation and insulin sensitivity. It serves as an essential regulator of adipocyte differentiation and lipid storage. Initially, roles were identified for PPARalpha in fatty acid catabolism. However, PPARalpha is also involved in sexual dimorphism especially by down-regulating gene expression. Using the steroid hydroxylase Cyp7b1 gene as a model, the molecular mechanism of this PPARalpha-dependent repression was elucidated. Initial sumoylation of the ligand-binding domain of PPARalpha triggers the interaction of PPARalpha with the GA-binding protein alpha (GABPalpha) bound to the Cyp7b1 promoter. A close by Sp1-binding site is methylated, which results in the loss of Sp1-binding and Cyp7b1 expression. Physiologically, this repression confers protection against estrogen-induced intrahepatic cholestasis.

Evidence has accumulated for an important role of PPARbeta in energy homeostasis, particularly in skeletal muscle. The activation PPARbeta induces a protection against diabetes and obesity. PPARbeta appears instrumental in the maintenance of oxidative fibers and fiber-type switching. Its invalidation leads to the metabolic disorders mentioned above.

Ablation of Pparbeta in the pancreas leads to hyperinsulinemia due to an increase of pancreatic beta-cell mass and insulin secretion. PPARbeta has a broad repressive function on the vesicular and granular compartment and actin cytoskeleton. Analyses of insulin release from isolated PPARbeta/delta-null islets revealed an accelerated second phase of glucose-stimulated insulin secretion. Taken together, these results provide evidence for a repressive role for PPARbeta in beta-cell mass and insulin exocytosis and shed new light on its metabolic action.

Healing of cutaneous wounds proceeds via a well-tuned pattern of events that include inflammation, re-epithelialization, and tissue remodeling. These events are regulated spatio-temporally by a variety of growth factors and cytokines. Activation of PPARbeta triggers keratinocyte survival and amplifies a cellular internal signal required for keratinocyte directional sensing and migration. In addition, IL-1 produced by the keratinocytes activates PPARbeta expression in the underlying fibroblasts, which in turn inhibits the mitotic activity of keratinocytes via inhibition of the IL-1 signaling pathway. These findings provide evidence for a novel homeostatic control of keratinocyte proliferation and differentiation, mediated via regulation by PPARbeta of sIL-1ra in dermal fibroblasts. Furthermore, PPARbeta is involved in skin tumor development after UV irradiation through the control of c-Src expression and downstream signaling pathways.

In conclusion, PPARs are implicated in important processes controlling cellular fate as well as in major metabolic and inflammatory regulations with obvious medical implications, especially related to metabolic diseases and tissue repair.

N. Leuenberger, S. Pradervand, W. Wahli (2009). Sumoylated PPARalpha mediates sex-specific gene repression and protects the liver from estrogen-induced toxicity in mice.
J Clin Invest, 119, 3138-3148.

H. C. Chong, M. J. Tan, V. Philippe, S. Hwey Tan, C. Kun Tan, C. Wai Ku , Y. Yih Goh, W. Wahli, L. Michalik, N.S. Tan (2009). Regulation of epithelial - mesenchymal IL-1 signaling by PPARbeta/delta is essential for skin homeostasis and wound healing.
J Cell Biol, 184, 817-831.

Tuesday, June 22, 9 a.m.

Model membranes for deciphering lipid transport

P. Bassereau*, L. Berland, A. Callan-Jones, B. Goud, L. Johannes, J.F. Joanny, J.B. Manneville, P. Nassoy, J. Prost, M. Safouanne, W. Römer, B. Sorre, G. Toombes (alphabetic order)

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Endocytosis, exocytosis, membrane transport between intracellular compartments, virus or toxin entry or exit out of the cell, all these processes imply to deform membranes and to redistribute lipids and proteins. Membrane deformation mechanisms of cell membranes by proteins but also lipid sorting mechanisms are currently actively studied in the cell biology context. But, there is a long history of membrane physics, which can help to better address this question. For more than 30 years, physicists have worked on developing theories and in vitro systems in order to model cell membranes. Systems with different geometries and controlled composition are available, among them Giant Unilamellar Vesicles (GUV), allowing for a direct comparison with theoretical models. In this talk, I will first briefly review the fundamental bases of membrane physics relevant for studying membrane trafficking. In a second part, I will show how membrane curvature and interactions between proteins defo rming membranes and lipids can drive lipid sorting. In order to study the effect of membrane curvature, membrane nanotubes pulled from GUV with a controlled diameter (15-500 nm) are very convenient model systems. We will show that curvature-induced lipid sorting only occurs if the membrane is close to a demixing point. In addition, for these compositions, lipid sorting is further amplified when even a low fraction of lipids is clustered upon cholera toxin binding suggesting that lipid-clustering proteins may play an important role in curvature-induced sorting in biological membranes. Another toxin, the B-subunit of Shiga toxin, binding to its lipid receptor Gb3 has been shown to induce tubular invaginations, in absence of any other cellular machinery, both in vivo and in vitro. We will show that the invaginations induced by STxB are enriched in membrane bound toxins and Gb3, but also in sphingomyelin, and are depleted from DOPC. This phenomenon is enhanced close to demixing. Our study suggests that lipids interacting favorably with a membrane deforming protein or with its receptor can be co-sorted in curved structures, overriding the curvature-induced sorting effect.

Tuesday, June 22, 9.50 a.m.

Phospholipid metabolism at the level of molecular species: determinants of acyl chain remodeling of phosphatidylcholine in yeast

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In *Saccharomyces cerevisiae*, phosphatidylcholine (PC) is a very abundant membrane phospholipid. It is synthesized in the ER by the Cho2-Opi3-catalyzed methylation of phosphatidylethanolamine, or via the CDP-choline route. Whereas the biosynthesis of PC has been extensively characterized in yeast, relatively little is known about the metabolism that acts downstream of the synthesis of PC, including the acyl chain remodeling of PC. We have used electrospray ionisation tandem mass spectrometry (ESI-MS/MS) with the aim of studying PC metabolism at the level of the molecular species. Pulsing wild type and mutant yeast cells with stable isotope-labeled lipid precursors allowed the visualisation of the substrate use of the phospholipid biosynthetic enzymes in live yeast by ESI-MS/MS. After a 10 min pulse with deuterated methionine and choline, detection of the newly synthesized PC by ESI-MS/MS revealed that the two biosynthetic pathways produce the PC species in different ratios. In a *pct1* strain lacking the CDP-choline route, remodeling by acyl chain exchange is required for attaining the steady state species profile of PC (1,2). In a database search for enzymes catalyzing the acyl chain exchange of PC, we identified 120 candidate genes. The corresponding deletion strains were obtained, and double mutants with an additional knockout of the *PCT1* gene were produced. The most promising candidates were selected in a screen of the PC species composition, and their ability to remodel PC was tested. The roles of several gene products in PC remodeling including several phospholipases B, the glycerol-3-phosphate acyltransferase (Sct1p/Gat2p) and the acylCoA binding protein (Acb1p) will be highlighted.

References

1. *Boumann HA, Damen MJ, Versluis C, Heck AJ, de Kruijff B, de Kroon AI. The two biosynthetic routes leading to phosphatidylcholine in yeast produce different sets of molecular species. Evidence for lipid remodeling. Biochemistry 2003;42:3054-3059.*
2. *De Kroon AI. Review: Metabolism of phosphatidylcholine and its implications for lipid acyl chain composition. Biochim Biophys Acta 2007;1771:343-352.*

Tuesday, June 22, 11 a.m.

Regulation of lipin function and membrane biogenesis

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Regulation of lipid metabolism is essential for many aspects of cell physiology, including growth, differentiation, signalling and transport. Lipins represent a novel and conserved family of phosphatidic acid (PA) phosphatases responsible for the biosynthetic production of diacylglycerol, a precursor for (a) membrane phospholipids and (b)
triacylglycerol, an essential storage form of energy and fatty acids. Consistent with these fundamental functions, lipins are required for nuclear/ER membrane biogenesis and adipogenesis and variation of their levels in mice can cause lipodystrophy or diet-induced obesity.

Unlike many other lipid biosynthetic enzymes, lipins lack transmembrane domains. Even more surprisingly, lipins can be also found into the nucleus of both yeast and mammalian cells, where they can regulate gene expression. By using genetic and biochemical approaches in yeast we are addressing the regulation of the membrane recruitment and nuclear translocation of lipins and examine how these steps impact on lipid and membrane biogenesis.

Tuesday, June 22, 11.50 a.m.

Cardiolipin metabolism in health and disease

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The mitochondrial phospholipid cardiolipin plays an important role in cellular metabolism as exemplified by its involvement in mitochondrial energy production and apoptosis. Following its biosynthesis, cardiolipin is actively remodeled to achieve its final acyl composition. An important cardiolipin remodeling enzyme is tafazzin and mutations in the corresponding gene cause the X-linked recessive disorder Barth syndrome.

After the identification of Barth syndrome as a "phospholipid disorder" and the involvement of cardiolipin in apoptosis, cardiolipin research has intensified but also diversified considerably. We discuss the current state of knowledge with emphasis on the role of cardiolipin in Barth syndrome but also cover new areas of research including apoptosis and cancer research.

Wednesday, June 23, 9.00 a.m.

Essential cellular functions of cardiolipin as physiological modifiers of Barth syndrome

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Cardiolipin (CL) is the signature lipid of mitochondria and plays a critical role in mitochondrial function and biogenesis. The availability of yeast mutants blocked in CL synthesis has facilitated studies of the biological role of this lipid. Perturbation of CL synthesis leads to growth defects not only during respiratory growth but also under conditions in which respiration is not essential. This suggests that CL is required for essential cellular functions not associated with mitochondrial energy production. Consistent with this, CL plays a role in mitochondrial protein import, cell wall biogenesis, longevity, vacuolar function, and cell division. Perturbation of CL remodeling (substituting one acyl group for another on the CL molecule), resulting in aberrant CL species, leads to the severe genetic disorder Barth syndrome (BTHS), characterized by cardiomyopathy, skeletal myopathy, and neutropenia. The clinical presentation of BTHS is highly variable, even among patients with the identical mutation, indicating that physiological factors play a significant role in modifying the phenotype. Elucidating the functions of CL is expected to shed light on the role of this important lipid in BTHS and other disorders of mitochondrial dysfunction.

Wednesday, June 23, 9.50 a.m.

Gangliosides in cell-cell recognition and signalling

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Gangliosides, sialic acid-bearing glycosphingolipids, are expressed by all vertebrate tissues and cells, and are the major glycans of nerve cells. They are anchored to the plasma membrane through their ceramide lipids with their varied glycans extending into the extracellular space.

Through sugar-specific interactions with glycan binding proteins and lateral associations with signaling components on the plasma membrane, gangliosides function as receptors for cell-cell recognition and regulate cell surface signaling. Paradigms of ganglioside-mediated recognition and ganglioside-mediated control of cell signaling will be provided with emphasis on the roles of brain gangliosides in axon-myelin stability and axon regeneration in the mammalian nervous system.

Wednesday, June 23, 11.00 a.m.

Sterol carrier protein 2 involved in maturation of iNKT cells and their stimulation by endogenous lipids

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The repertoire of invariant Natural Killer T (iNKT) cells is determined by production and presentation of endogenous lipid antigens, which remain unknown. We find that mice lacking the lipid transfer protein SCP-2 have a reduced number of iNKT cells in the thymus and in peripheral organs. Residual iNKT cells from *Scp2*$^{-/-}$ mice are functionally normal as are the antigen-presentation capacities of thymocytes and dendritic cells, thus excluding intrinsic alterations of iNKT antigen recognition and of lipid antigen presentation. Importantly, endogenous lipids of *Scp2*$^{-/-}$ mice differ from those of wild-type mice and stimulate iNKT cells less efficiently than endogenous lipids from wild-type mice, suggesting that alterations of lipid repertoire may also affect the endogenous lipid antigens stimulating iNKT cells. The nature and generation of the stimulatory lipids will be discussed.

Wednesday, June 23, 11.50 a.m.

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.

Wednesday, June 23, 12.10 a.m.

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.

Wednesday, June 23, 12.30 a.m.

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.