workflow. Neutral loss scanning, including multistage activation, on an LTQ-orbitrap mass spectrometer is an alternative to the precursor ion scanning used on triple quadrupole mass spectrometers. This high resolution and high mass accuracy mass spectrometer can be used successfully for the identification of protein phosphorylation sites and the identification sites in the protein UCP1 will be used as an example.

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S16/3 Mammalian liver cytochrome *c* is tyrosine-48 phosphorylated *in vivo*, inhibiting mitochondrial respiration

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Cytochrome *c* (Cyt *c*) is part of the mitochondrial electron transport chain (ETC), accepting electrons from bc_1 complex and transferring them to cytochrome *c* oxidase (CcO). Considering its central role in life (respiration) and death (apoptosis) decisions one would expect tight regulation of Cyt *c* function. We have recently shown that Cyt *c* isolated from cow heart tissue is phosphorylated on tyrosine 97 *in vivo*, which leads to inhibition of respiration in the reaction with CcO. In this study we isolated Cyt *c* from a different organ, cow liver, under conditions preserving the physiological phosphorylation state. Western analysis with a phospho-tyrosine specific antibody suggested that liver Cyt *c* is phosphorylated. Surprisingly, the phosphorylation site was unambiguously assigned to Tyr-48 by immobilized metal affinity chromatography/nano-liquid chromatography/electrospray ionization mass spectrometry, and not to the previously identified phospho-Tyr-97 in cow heart. As is true of Tyr-97, Tyr-48 is conserved in eukaryotes. As one possible consequence of Tyr-48 phosphorylation we analyzed the *in vitro* reaction kinetics with isolated cow liver CcO revealing striking differences. Maximal turnover of Tyr-48 phosphorylated Cyt *c* was 3.7 s^{-1} whereas dephosphorylation resulted in a 2.2 fold increase in activity to 8.2 s^{-1}. Effects of Tyr-48 phosphorylation based on the Cyt *c* crystal structure are discussed.

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S16/4 Mitochondrial comparative proteomics: Strength and pitfalls

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In this review, we describe the various techniques available to carry out valid comparative proteomics, their advantages and their disadvantages according to the goal of research. The two-dimensional gel electrophoresis and the 2D-DIGE (Differential in-gel electrophoresis) are compared with the shotgun proteomics and SILE (Stable isotopic labeling experiments). We deliver our opinion on their best fields of application in the domain of the comparative proteomics. We underline the utility of these new tools, providing mass data to study physiology and mitochondrial plasticity in front of a specific mitochondrial insufficiency or exogenous stresses. We illustrate our matter with results obtained in our laboratory specifying the importance of an approach of comparative proteomics combined on mitochondria and the cell which makes it possible to obtain important information on the statute of the mitochondrial function at the cellular level. Finally, we draw attention to the dangers of the extrapolation of the data of proteomics to metabolic flows which require the greatest care.

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(S16) Proteomics and mitochondria symposium abstracts (poster and raised abstracts)

S16.5 High sensitivity identification of membrane proteins by MALDI TOF-mass spectrometry using polystyrene beads

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Uncoupling proteins (UCPs) are transporters present in the inner membrane of mitochondria. They are found in all mammals and in plants, and they belong to the family of anionic mitochondrial carriers. Their roles are quite well established for UCP1 in nonshivering thermogenesis process, but are still in debate for the others UCPs. We study theses proteins by biophysical approaches such as mass spectroscopy (MS). Peptide mass fingerprinting methods using techniques such as MALDI-TOF MS, for example, have become an important analytical tool in the identification of proteins. However, PMF of membrane proteins is a real challenge for at least three reasons. First, membrane proteins are naturally present at low levels; second, most of the detergents strongly inhibit proteases and have deleterious effects on MALDI spectra; and third, despite the presence of detergent, membrane proteins are unstable and often aggregate. We improved and showed that differential acetonitrile extraction of tryptic peptides combined with the use of polystirene Bio-Beads triggered high resolution of the MALDI-TOF identification of mitochondrial membrane proteins solubilized either with Triton-X100 or CHAPS detergents. The sequence coverage of UCP1 obtained by this approach is close to 90% and allows further investigations for characterizing regulators bound to UCP1.

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S16.6 Analysis of synaptic and non-synaptic mitochondria using colorless- and blue-native PAGE

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Mitochondrial dysfunction contributes to a wide range of human diseases, including neurodegenerative diseases. Proteomic technology can give novel insights into the structure and composition of the brain mitochondrial electron transport chain (ETC) complexes. Using beef heart mitochondria as a control and reference point, this study reports a comparison of rat synaptic and non-synaptic mitochondrial protein profiles using blue-native and colorless-native (CN) gel electrophoresis combined with Tricine-SDS PAGE and MALDI-TOF mass spectrometry. BN-PAGE was found to be a straightforward tool for proteomic analysis of ETC complexes (I, III, IV and V) and especially for the identification of very hydrophobic membrane protein constituents that are not accessible by common isoelectric focusing/sodium dodecyl sulphate gel electrophoresis. The introduction of CN-PAGE into a three dimensional electrophoresis greatly improved the isolation and

resolution of the ETC complexes in brain mitochondria. The protein complexes of the mitochondrial oxidative phosphorylation system have been reported to form supramolecular assemblies termed respiratory supercomplexes or respirasomes. BN-PAGE was used in this study to analyze the mitochondrial subunit assembly into respiratory chain complexes in rat brain synaptic and non-synaptic mitochondria. Using the mild detergent digitionin for solubilisation of mitochondrial membranes, it was shown that complexes I and II–V interact to form supercomplexes. However, initial experiments suggest that the supercomplex composition is different between synaptic and non-synaptic mitochondria from rat brain. The consequences for such disparity in supercomplex formation will be discussed.

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S16.7 Analysis of proteins released through the permeability transition pore of rat brain mitochondria

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Mitchondrial dysfunction can contribute to cell death by not only energetic failure and increased ROS production, but also by the activation of the mitchondrial permeability transition pore (PTP) and release of proapoptotic proteins. The PTP complex is a dynamic polyprotein complex, which spans both mitochondrial membranes at the contact site. An elevation of matrix calcium, beyond a critical threshold, is one of the strongest inducers of the pro-apoptotic PTP. Differing flux control coefficients and energy thresholds have been recorded between synaptic and nonsynaptic mitochondria extracted from rat brain, however, little is known about the proteins that are released from their respective PTPs under stressful conditions. In this study we investigated the calcium-induced swelling in energized/de-energized synaptic and non-synaptic rat brain mitochondria. We report that rat brain PTP opening (as measured by swelling) in both types of mitochondria was more sensitive to Bongkrekic acid than to Cyclosporin A. Furthermore, following swelling of the mitochondria, the proteins released through the pore were resolved on a 2D-PAGE and identified by MALDI-TOF mass spectrometry. The differences between the proteins released through the PTPs from synaptic and non-synaptic rat brain mitochondria and their physiological implications will be discussed.

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(C1) Genomics and evolution colloquium lecture abstracts

C1/1 Introductory notes: Energetic constraints at the very beginning of life

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The number of hypothetical scenarios for the origin of life is unlimited. The space of possibilities, however, can be dramatically restricted by consistently invoking physical, chemical, biological and geological constraints. The short introductory talk will focus on energetic constrains, in particular on the consideration of energy sources that could be available and utilizable at the earliest stages of evolution.

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C1/2 Energetics of the first bacteria as inferred from genome analysis

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The availability of complete genome sequences had a major impact on modern biology, resulting in a much better understanding of cell metabolism. Owing to their complex subunit structure, membrane energy-transducing complexes remained outside the scope of most comparative-genomic analyses. We compared the distribution of proton- and sodium-translocating enzymes encoded in bacterial and archaeal genomes, analyzed the physico-chemical and evolutionary constraints for their origin, and used these data to infer an evolutionary scenario for the origin of the energy transduction machinery. Surprisingly, results of comparative structural and phylogenetic analyses suggest that sodium-translocating ATP synthases and ion pumps preceded the proton-translocating ATP synthases and proton pumps. Thus, the first prokaryotes likely relied on sodium ion gradient for their energy metabolism. Proton-based energetics must have emerged later, following the development of proton-tight membranes through different adaptations in bacteria, archaea and eukaryotes. Evolutionary advantages of proton-based energetics, in particular, chemical coupling of transmembrane proton translocation with electron transfer from organic substrates to terminal electron acceptors, such as oxygen or nitrate, ensured wide dissemination of the corresponding genes and resulted in the switch from Na^+ to H^+ as the coupling ion in most bacteria and archaea. Currently, sodium-based energetics is found primarily in obligate anaerobic prokaryotes, including some important human pathogens.

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C1/3 Chloroplast sensor kinase — The redox messenger of organelle gene expression

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Why are there genes in chloroplasts and mitochondria? The CoRR hypothesis states that organellar genes and their gene products are Co-located for Redox Regulation. CoRR predicts (i) that an irreducible core of genes must be retained by chloroplasts and mitochondria from their bacterial ancestors, and (ii) that a bacterial redox signalling pathway exerts regulatory control over expression of these genes, using components that have operated continuously throughout the transition from prokaryote to bioenergetic organelle. Chloroplast Sensor Kinase (CSK) is a chloroplast stromal protein that is the product of the nuclear gene *At1g67840* of *Arabidopsis thaliana*. T-DNA insertion lines are impaired in plastoquinone redox control of transcription of chloroplast genes for reaction centre apoproteins of photosystem I and II and do not adjust PS I/PS II stoichiometry. CSK is homologous with bacterial histidine sensor kinases and yet is universal in photosynthetic eukaryotes. We propose that CSK provides the redox regulation