importance of the GTPase domain and behavior during apoptosis. Using immunofluorescence, we found that endogenous Mfn2 is enhanced in specific regions on the mitochondria, particularly at tips of mitochondria. Overexpressed mitofusin also forms distinct foci and we have found using FRAP (Fluorescence Recovery After Photobleaching) that mutants of mitofusins devoid of GTP binding display markedly different mobilities on the mitochondrial membrane reflecting changes in complex formation. During apoptosis the mitochondria fragment and fusion is inhibited. Interestingly, during apoptosis a mutant of Mfn2 that typically is incapable of forming foci moves into foci that coalesce with Bax, the pro-apoptotic Bcl-2 family member. Curiously, we have found that upon Bax translocation the mitofusins, along with Fis1 and OMP-25, lose mobility, indicating that the mitochondria outer membrane is undergoing additional changes not previously described.

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S9.9 MiD51 and MiD49: New mediators of mammalian mitochondrial distribution

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Mitochondria are organized into networks that are important for proper cellular function. These networks are regulated by fission and fusion events, as well as transport along cytoskeletal elements. The aim of this study was to characterize two novel mitochondrial proteins, *Mi*tochondrial *D*istribution protein 51 kDa (MiD51) and 49 kDa (MiD49), that appear to be involved in the regulation of mitochondrial morphology and distribution. Ectopic expression of both MiD51 and MiD49 in COS-7 cells results in two aberrant mitochondrial phenotypes — long extended tubules and peri-nuclear collapsed mitochondria. Live cell confocal microscopy was used to highlight the dynamic nature and connectivity of the mutant mitochondria by co-expression with the photoswitchable fluorescent protein mitochondrial-Dendra2. While mitochondrial movement in mammalian cells is principally driven by a connection with the microtubule network, these MiD proteins instead appear to induce an interaction between mitochondria and actin filaments. Only simultaneous knockdown of both endogenous proteins via RNAi, results in atypical mitochondrial distribution in both COS-7 and HeLa cells, as well as reduced HeLa cell viability. We therefore propose that MiD51 and MiD49 share functional redundancy in the distribution of mitochondria in mammalian cells, perhaps in regulation of actin/mitochondrial interactions.

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S9.10 Cell type specificity of mitochondrial dynamics: Striking differences between adult rat cardiomyocytes, HL-1 cells and human pancreatic cells

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The aim of this study was to analyze mitochondrial dynamics in adult rat cardiomyocytes, HL-1 cells and human pancreatic cells. Mitochondrial imaging was performed by real time confocal microscopy using mitochondria-specific fluorescent probes. The results revealed remarkable differences in mitochondrial dynamics, as well as in spatial arrangement of mitochondria in these cells, probably due to cell specific cytoskeleton organization. In adult rat cardiomyocytes, in which mitochondria are arranged regularly (crystal-like), no displacement of mitochondria was observed with only very small amplitude rapid vibration. In contrast, in primary human pancreatic and HL-1 cells we documented complex dynamic behaviour of mitochondria. The common types of mitochondrial dynamics observed were: 1) fission, fusion and small oscillatory movements of mitochondria; 2) larger movements including filament extension, retraction, and 3) fast oscillating branching in the mitochondrial network and fast long-distance intracellular translocation of single mitochondria or mitochondrial filaments. In summary, we show that mitochondrial dynamics may be very different in different cell types. These variations could be related to a significant role of cell specific integrations of mitochondria with other intracellular systems like cytoskeleton and ER.

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S9.11 Novel mechanism of elimination of malfunctioning mitochondria (mitoptosis): Formation of mitoptotic bodies and extrusion of mitochondrial material from the cell

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Energy catastrophe when mitochondria hydrolyze glycolytic ATP instead of producing it has been modeled. In highly glycolyzing HeLa cells 30–50% of the population survived after inhibition of respiration and uncoupling of oxidative phosphorylation for 2–4 days. The survival was accompanied by selective elimination of mitochondria. This program of mitoptosis includes (i) fission of mitochondrial filaments, (ii) clusterization of mitochondria in perinuclear area, (iii) occlusion of mitochondrial clusters by a membrane (formation of a "mitoptotic body"), (iv) decomposition of mitochondria to small vesicles, (v) protrusion of the body from the cell and (vi) disruption of the body boundary membrane. Autophagy was not involved in mitoptosis. Increased production of reactive oxygen species (ROS) was necessary for execution of the program, since antioxidants prevent mitoptosis and kill the cells treated with the mitochondrial poisons. Mitoptosis served for protection of the cells under the conditions of severe damage of mitochondria. It is suggested that exocytosis of mitoptotic bodies may be involved in maturation of reticulocytes and precursors of lens fiber cells.

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(S10) Mitochondria and reactive oxygen containing species symposium lecture abstracts

S10/1 Interactions of nitric oxide with cytochrome *c* and cytochrome *c* oxidase

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At low nM concentration nitric oxide (NO) is an intercellular messenger, interacting with the heme protein guanylate cyclase.

However, under a range of pathological conditions NO levels can rise to micromolar levels and disrupt a wide range of cellular processes. Between these two extreme concentration ranges NO can interact with mitochondria in ways that have been described as physiological or pathophysiological. The highest affinity target is cytochrome *c* oxidase. This talk will review this interaction and suggest that it has the potential to play a role in both NO signalling and detoxification. NO has also been shown to interact with lower affinity with mitochondrial cytochrome *c* in both its reduced and oxidised forms. Previous studies have indicated that NO can oxidise cytochrome *c* at physiological pH. We show that most of this oxidation is not caused by direct reactions of NO, but instead by a reaction product of NO and oxygen, most likely NO_2. In contrast the reaction of NO with oxidised cytochrome *c* is a direct, reversible binding. Intriguingly in the presence of cardiolipin NO can also bind reduced cytochrome *c* with high affinity at neutral pH. We will describe these interactions and comment on their biological relevance.

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S10/2 Mitochondria and reversible apoptosis

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Apoptosis has been considered as a form of cell death, to which a cell is irreversibly committed once cytochrome *c* is released from the mitochondria. However, apoptosis can also be regarded as a means of signalling to phagocytes, which may in principle be reversible up to the point of phagocytosis. We find that mitochondrial cytochrome *c* release does not inevitably commit a cell to death because if the cell reduces the cytosolic cytochrome *c* then caspase activation is blocked. Similarly caspase activation does not inevitably commit a cell to death, because the caspases can be inactivated by endogenous oxidants. Phosphatidylserine flip to the outer leaflet of the plasma membrane is also a reversible process, as long as phagocytes are not present to eat the cell. We find that many aspects of apoptosis are fully reversible in neurons. This leads to the conclusion that apoptosis can (in some circumstances) be reversible and is not always a form of cell death.

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S10/3 Ischemic preconditioning invokes multiple mechanisms of nitric oxide and reactive oxygen signaling at the mitochondrial level

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Ischemic preconditioning (IPC) is a phenomenon in which short cycles of ischemia and reperfusion (IR) can protect organs such as the heart and brain from prolonged IR injury. Mounting evidence suggests that both mitochondria and nitric oxide play critical roles in IPC signaling, although the exact biochemical mechanisms underlying cardioprotection are poorly understood. We have identified 3 novel biochemical events involving reactive oxygen and nitrogen species, which may contribute to the modulation of mitochondrial function in IPC: (1) *S*-nitrosation and reversible inhibition of respiratory chain complex I. (2) Activation of mild uncoupling via the generation of NO derived electrophilic lipids (nitro-alkenes) and their post-translational modification of mitochondrial carrier family proteins. (3) Endogenous generation of mitochondrial K^+_{ATP} channel agonists and complex II inhibitors, via redox reactions involving Krebs' cycle intermediates. Together, it is thought that these 3 pathways all act to diminish mitochondrial Ca^{2+} overload and ROS generation at reperfusion, thereby limiting the opening of the permeability transition pore. A brief outline of the key findings in support of these novel signaling pathways will be discussed.

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(S10) Mitochondria and reactive oxygen containing species symposium abstracts (poster and raised abstracts)

S10.4 Mild uncoupling reduces oxidative stress in intact cells

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"Mild uncoupling", the introduction of a limited proton conductance into the mitochondrial inner membrane, has been demonstrated to greatly reduce reactive oxygen species (ROS) production in isolated mitochondria. On the other hand, it will also increase energy consumption to maintain the mitochondrial membrane potential, and cells need energy in the form of NADH to maintain the cellular protein machinery in the normal, reduced state. For this reason it has been a subject to debate whether mild uncoupling is a viable strategy to reduce oxidative stress in intact cells and ultimately in vivo, or whether the increased energy expenditure might have detrimental effects. Using a redox-sensitive green fluorescent protein (roGFP1) targeted to mitochondria of PC12 cells, we found that mild uncoupling using low concentrations of chemical uncouplers FCCP or DNP indeed reduced oxidative stress, whereas strong uncoupling caused oxidation of roGFP1, indicating that the beneficial effects of uncoupling are limited to a certain range of membrane potential, and that uncoupling beyond this range will increase oxidative stress probably due to energy crisis. This leads to the conclusion that while mild uncoupling might be beneficial under some circumstances, it is a dangerous strategy since the safe range of uncoupling will probably depend on cell type and metabolic state.

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S10.5 The role of reactive oxygen and nitrogen species in the pathogenesis of acute renal failure

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The aim of this work was to study mechanisms of acute renal failure (ARF) development in kidney survived ischemia or mioglobinuria (rabdomyolysis). Experimental model of those pathologies showed that 3 days after ischemia or induction of rabdomyolysis, the levels of urea, creatinine and cell death markers increase. Such kidney malfunctioning can be caused by tubular epithelium destruction. In our experiments, lipid peroxidation products accumulated in kidney and total antioxidant activity of blood are decreased. Also the level of nitrite in blood serum was increased indicating the activation of NO production. Analysis of ROS- and NO-production in kidney cells revealed amplification of the production of these radicals. We