functional coupling. The pH spikes were abrogated by protonophores and their frequency strongly decreased by respiratory chain inhibitors (rotenone: -57%, antimycin: -96%) or by inhibition of the ATP-synthase (oligomycin: -52%). Conversely, inhibition of the adenine nucleotide exchanger (ANT) by actractyloside increased spike frequency by 510%. Normal pH spikes were observed in cells pre-treated with the SERCA-ATPase inhibitor thapsigargin, indicating that the pH elevations did not require calcium release from intracellular stores. Simultaneous ψ_{mt} and pH_{mito} measurements revealed concomitant depolarization and basification transients. Superoxide flashes with similar properties were previously reported in individual mitochondria with a circularly permutated YFP (Wang *et al.*, 2008, *Cell* 134). Since this probe is known to be pH-sensitive, the signals reported as superoxide flashes might have been due to pH spikes. Alternatively, superoxide flashes could generate pH spikes *via* Fenton and dismutation reactions, although we did not detect ROS elevations with the mitochondrial ROS sensor roGFP. In summary, we show that individual mitochondria in intact HeLa cells undergo spontaneous basification transients. The pH spikes are not due to calcium release from stores, but require functional OXPHOS machinery.

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15L.4 Mitochondrial cholesterol and cell death

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Cholesterol is a critical component of biological membranes, which determines their structural and biophsycial properties. Its distribution within membranes is heterogeneous, partitioning in specialized domains called rafts, where modulate signaling pathways. Due to this fundamental role cholesterol levels are highly regulated. Cholesterol distributes to different subcellular compartments by vesicular dependent and independent mechanisms. Compared to plasma membranes, mitochondria are cholesterol-poor organelles, with estimates of 0.5-3% of the total cholesterol pool. While hepatic mitochondrial cholesterol plays an important physiological role such as in the synthesis of bile acids, its accumulation contributes to liver diseases, such as alcoholic (ASH) and non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC). Mitochondrial cholesterol loading in ASH and NASH models sensitizes hepatocytes to oxidative stress and inflammatory cytokines, contributing to fatty liver disease by a mechanism that involves mitochondrial GSH (mGSH) depletion due to changes in mitochondrial membrane dynamics. mGSH depletion protects cardiolipin from oxidation to peroxidized cardiolipin, which determines mitochondrial membrane permeabilization by proapoptotic bcl-2 family members, such as Bax. Interestingly, mitochondrial cholesterol accumulation also occurs in HCC, which contributes to chemotherapy resistance. However, despite cholesterol loading, HCC cells exhibit unimpaired transport of GSH into mitochondrial matrix due to the overexpression of mGSH carriers, 2-OG and DIC. This maintenance of mGSH prevents cardiolipin peroxidation. Peroxidized cardiolipin, however, overcomes the resistance to mitochondrial membrane permeabilization induced by Bax. These results characterize mitochondrial cholesterol/peroxidized cardiolipin as a rheostat in cell death regulation.

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15L.5 Signalosomes transmit signals from plasma membrane receptors to mitochondria

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When an agonist binds to a plasma membrane receptor, a signaling cascade is triggered that targets intracellular organelles. Mitochondria respond by opening the mitochondrial ATP-sensitive K^+ channel ($mitoK_{ATP}$) and producing ROS for further signaling. Cardioprotection against ischemia- reperfusion (IR) injury is a useful experimental model for probing this process, because these signals reduce infarct size by about 70%. Many receptors produce the cardioprotective response, including Gi protein-coupled receptors (adenosine, acetylcholine, bradykinin, opioids, and phenylephrine), the Na,K-ATPase (ouabain, digitalis), and the L-type Ca^{2+} channel (Ca^{2+}). We have found that the entire signaling cascade is assembled in plasma membrane caveolae, then buds off as a 140 nm signalosome, internalizes, and migrates to mitochondria. The terminal kinases of the cascade phosphorylate a protein on the outer membrane. This leads to activation of an inner membrane PKCε, which opens $mitoK_{ATP}$ by phosphorylation. The signalosomes can be isolated and purified from the perfused heart and displays activity *in vitro*. This allows us to study a signaling unit in its naturally organized state with preserved functionality. Most signalosomes are functionally active within minutes of receptor activation. Interestingly, the adenosine signalosome requires an additional step of ROS activation after internalization, and the adenosine receptor remains active *in vitro*.

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15L.6 The gas pedal of brain mitochondria: glutamate supply for OXPHOS is fully regulated by cytosolic Ca^{2+} *via* activation of aralar

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The regulation of OXPHOS is still not understood in detail. ADP formed by ATP-consuming enzymes activates OXPHOS but in the heart cytosolic ADP is only insignificantly increased *in vivo* during elevated work loads [1] and therefore the parallel stimulation of OXPHOS and work load by cytosolic Ca^{2+} (Ca^{2+}_{cyt}) was assumed [2]. However, activation of dehydrogenases by matrix Ca^{2+} [3] complies only partially with the *in vivo* findings, therefore we hypothesized that other mechanisms should be responsible for mitochondrial activation by Ca^{2+}_{cyt}. We have found recently [4-6] that the glutamate dependent respiration of brain mitochondria can be stimulated by Ca^{2+}_{cyt} due to the activation of aralar [7], the glutamate aspartate carrier ($S_{0.5}$ = 260 nM Ca^{2+}_{free}). Depending on its initial concentration, Ca^{2+} can activate state $3_{glu/mal}$ of brain mitochondria up to

600%. Kinetic analyses revealed that this activation occurs by increasing V_{max} but the K_M for glutamate remains unchanged (0.6 mM glutamate at 10 nM Ca^{2+}, 0.7 mM glutamate at 757 nM Ca^{2+}). In contrast the activation of α-KGDH by *intra*mitochondrial Ca^{2+} is caused by increasing affinity to the substrate (1.4 mM α-KG at 10 nM Ca^{2+}, 0.4 mM α-KG at 757 nM Ca^{2+}) but the V_{max} is not changed as is known also from earlier work [2]. With decreasing Ca^{2+} the activity of aralar diminishes and the supply of mitochondria with reducing hydrogen from NADH decreases too. Aspartate titrations revealed a large control of aralar over oxidative phosphorylation. Moreover decreasing Ca^{2+} shifts the force/flow relation of state $3_{glu/mal}$ to lower DY-values. Whereas Ca^{2+} activation of α-KGDH changes the turnover speed at low substrate concentrations, which are inside the matrix space, Ca^{2+} activation of aralar regulates the substrate supply to mitochondria. Therefore we conclude that brain mitochondria together with the cytosolic enzymes of malate aspartate shuttle act as a mitochondrial "gas pedal" with Ca^{2+}_{cyt} as physiological controller regulating the substrate supply on demand.

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15L.7 Calcium signalling and ER-mitochondrial communication

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The ER-mitochondrial junction provides a local calcium signaling domain that is critical for both matching energy production with demand and the control of apoptosis. Here, we describe a novel genetic approach to visualize ER-mitochondrial contact sites and to monitor the localized [Ca^{2+}] changes in the narrow space between ER and mitochondria ([Ca^{2+}]$_{ER\text{-}mt}$) using drug-inducible fluorescent interorganellar linkers. We show that essentially all mitochondria have contacts with the ER in both RBL-2H3 and H9c2 cells. Plasma membrane-mitochondrial contacts are less frequent because of interleaving ER stacks. Single mitochondria display discrete patches of ER contacts. Cytoplasmic and mitochondrial matrix [Ca^{2+}] showed robust ER-mitochondrial Ca^{2+} transfer with considerable heterogeneity even among adjacent mitochondria. Pericam-tagged linkers revealed IP_3-induced [Ca^{2+}]$_{ER\text{-}mt}$ signals that exceeded 9 mM and were resistant to buffering bulk cytoplasmic [Ca^{2+}] increases. Lengthening the linker to span the space required for the Ca^{2+} transfer machinery, positively affected the [Ca^{2+}]$_{ER\text{-}mt}$ signals. These studies provide direct evidence for the existence of high Ca^{2+} microdomains between the ER and mitochondria, and indicate their biphasic dependence on the ER-mitochondrial gap width.

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15L.8 The mitochondrial permeability transition pore: from molecular mechanism to cardioprotection

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Opening of the mitochondrial permeability transition pore (MPTP) plays a key role in mediating the damage to the heart that occurs following ischaemia and reperfusion (reperfusion injury) [1]. Reactive oxygen species (ROS) play a key role in triggering MPTP opening and new data on the involvement of cytochrome *c* in regulating ROS production will be described. Inhibition MPTP opening provides powerful protection against reperfusion injury and understanding the molecular mechanism of the MPTP is important for the design of new cardioprotective drugs. However, the true molecular identity of the MPTP remains uncertain [2]. Knockout studies have confirmed regulatory roles for cyclophilin-D (CyP-D) and the adenine nucleotide translocase (ANT). The former facilitates a conformational change in the pore forming protein within the inner membrane and is mediated by its peptidyl-prolyl cis-trans isomerase activity. The ANT mediates the inhibitory effects of adenine nucleotides on MPTP opening. Indeed, the oxidation of specific thiol groups on the ANT overcomes this inhibition and accounts, in part, for the powerful activation of MPTP opening by oxidative stress. More recently we have demonstrated a critical role for the mitochondrial phosphate carrier (PiC) in MPTP opening and shown that the PiC is the major inner membrane protein to bind CyP-D [2]. We have used knockdown of the PiC by siRNA to confirm its role in MPTP formation but these studies have raised problems. First, it is difficult routinely to achieve greater than 80% PiC knockdown. Second, the use of the calcium retention assay to measure MPTP opening in permeabilised cells is not appropriate when phosphate transport into mitochondria is prevented since calcium entry into mitochondria is dependent on coincident uptake of Pi. Progress in circumventing these technical problems to reveal whether or not the PiC is essential for MPTP formation will be described.

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15L.9 Novel protein partners in the control of the brain mitochondrial permeability transition: 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and p42^{IP4} (centaurin α1; ADAP 1)

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Increase of permeability of the inner mitochondrial membrane in response to threshold calcium concentration or oxidative stress leads to the formation of an unselective permeability transition pore (PTP) complex. In spite of intense studies of the PTP phenomenon, the structure of the pore complex is still unknown. Earlier accepted involvement of voltage-dependent anion channel and adenine