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Regulation of Mitochondrial Motility by Milton-like Proteins, OIP106 and GRIF1

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GRIF1 and OIP106 are mammalian homologs of Milton, a kinesin-binding protein that forms a complex with Miro GTPase, an integral outer mitochondrial membrane EF-hand protein. Kinesin, Milton and Miro have been proposed to function together in the movement of mitochondria along the microtubules. We report here the influence of overexpression of OIP106 and GRIF1 on mitochondrial motility and its inhibition by agonist-induced cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) signals in H9c2 cells. HA-tagged OIP106 and GRIF1 were overexpressed at similar level and were localized to the mitochondria as detected by immunocytochemistry. In OIP106 and GRIF1 overexpressing cells, elongated mitochondria with varied degree of aggregation were visualized both in live and in fixed, immunostained samples. Mitochondrial motility at resting Ca^{2+} (< 100nM) was greatly enhanced by OIP106 and to a lesser extent by GRIF1 (26.6± 2.3 and 21± 1.9 motility units, respectively against a control value of 14.9±1.4 motility units). Furthermore, the Ca^{2+}-dependent inhibition of motility during stimulation by vasopressin (100nM) was suppressed by overexpressed OIP106 (40.0±3.2%), whereas GRIF1 (52.1± 4.3%) had no significant effect compared to control (58.0±3.3%). Overexpression of OIP106 or GRIF1 did not alter either basal $[Ca^{2+}]_c$ or agonist-induced $[Ca^{2+}]_c$ levels measured by fura2. Collectively, these data show that both OIP106 and GRIF1 can modulate mitochondrial motility presumably, by promoting the association of mitochondria with the motor proteins. Furthermore, OIP 106 seems to have greater efficacy in the control of mitochondrial movements.

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Mitochondrial localization and function relationship

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Mitochondrial contribution to cell signaling and function relies on the association and local communication of mitochondria with the ER and the plasma membrane (PM). We have recently shown that the local Ca2+ communication between ER and mitochondria is supported by interorganellar tethers, and created synthetic linkers that connected the outer mitochondrial membrane (OMM) to the ER and sensitized mitochondria to ER Ca2+ release. To study the kinetics and short-term effects of the linkage formation, we have now devised fluorescent protein pairs targeted to the OMM and ER or PM containing FKBP12 or FRB domains. Rapamycin causes heterodimerization of these proteins to form OMM-ER or OMM-PM bridges. Confocal imaging of the inducible fluorescent linker pairs revealed increased association of ER or PM-patches with the mitochondria within minutes of rapamycin exposure. The linkage formation between the ER and mitochondria was followed by a decrease in mitochondrial motility (>40% in 15 min) and by sensitization of mitochondria to IP_3 receptor-mediated Ca2+ release. Linkage formation between PM and mitochondria also suppressed mitochondrial motility. The initial kinetics and spatial distribution of linkage formation could be followed via recording FRET between CFP-and YFP-containing linker partners. A longer version of the linker was found to show faster increase of the FRET signal, supporting the idea that the rate of linkage formation positively correlates with the tightness of the ER-mitochondrial contacts. Currently, we are using the FRET kinetics to visualize the areas of close ER-mitochondrial interface and study its relevance in the Ca2+ signal propagation to the mitochondria. Thus, inducible interorganellar linkers provide a tool to assess the distance between organelles in live cells, to establish rapid changes in the subcellular distribution and dynamics of the organelles and to evaluate the ensuing changes in organellar and cell function.

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Bak/bax-dependent Apoptotic Signaling In Vdac2$^{-/-}$ Cells

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Bid, a pro-apoptotic Bcl-2 family protein, upon activation forms truncated Bid (tBid) that binds to the outer mitochondrial membrane (OMM) and engages Bak/Bax-dependent release of cytochrome c (cyto c) and other intermembrane space proteins from mitochondria to the cytosol to induce apoptosis. The voltage-dependent anion channel (VDAC) is the major permeability pathway for metabolites and ions in the OMM but its role in the tBid-induced OMM permeabilization remains controversial. Previously we reported that among the VDAC isoform-specific knockout mouse embryonic fibroblasts (MEFs), only VDAC2$^{-/-}$ MEFs lack tBid induced complete cyto c release and loss of $\Delta\Psi$m. Here we show by single cell fluorescence imaging that permeabilized VDAC2$^{-/-}$ MEFs expressing cyto c-GFP were resistant to tBid (37nM)-induced cyto c-GFP release. Furthermore, by rescuing VDAC2$^{-/-}$ MEFs with VDAC2 the tBid-induced cyto c-GFP release was restored. In addition, tBid adenovirus infection caused less cell death in intact VDAC2$^{-/-}$ MEFs than in wildtype (WT) MEFs. It has been reported that VDAC2 is required for proper targeting of Bak in OMM. Indeed, Bak did not appear in the membrane fraction of VDAC2$^{-/-}$ MEFs. Along this line we show that permeabilized Bak$^{-/-}$ cells were more resistant to tBid induced cyto c release and loss of $\Delta\Psi$m than WT and Bax$^{-/-}$ MEFs. Strikingly, washout of the cytosol further desensitized the Bak$^{-/-}$ MEFs to tBid. Unlike VDAC2$^{-/-}$ MEFs, the Bak$^{-/-}$ MEFs constitutively overexpressed Bax that was primarily localized in the cytosol. However, recombinant Bax (200nM) could induce cyto c release and depolarization in VDAC2$^{-/-}$ MEFs and also supported the tBid-induced cyto c release. Thus, in VDAC2$^{-/-}$ cells Bak does not localize to the mitochondria and fails to interact with tBid and does not allow a compensatory increase in Bax. The combination of these two mechanisms greatly attenuates tBid-induced OMM permeabilization and apoptosis.

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Mitochondrial fusion-fission dynamics during hypoxia/reoxygenation

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Structural, biochemical, and functional abnormalities of mitochondria during hypoxia/reoxygenation (H/R) are widely believed to be important pathogenic factors that underlie cell injury. However, mitochondrial fusion-fission dynamics responses to H/R are unclear. We investigated the effect of H/R and chemical hypoxia evoked by KCN on cellular ATP, $\Delta\Psi_m$, and on mitochondrial morphology and fusion. Sixty min H caused cellular ATP decrease to 71±3%. $\Delta\Psi_m$ showed progressive decrease during H, gradually improved in the first 30 minutes R and decreased again during longer R. Mitochondrial fusion activity decreased to 65% during H and to 58% during R. In addition, anomalous fusion (autofusion and fusion at multiple sites among 2-3 mitochondria) produced donut-shaped mitochondria during R. Cyclosporine A (CSA), an inhibitor of the permeability transition pore (PTP) relieved the fusion inhibition (70%) and prevented donut formation during R. Five mM and 10 mM KCN could induce cellular ATP decrease to 49±6% and 23±2%, and $\Delta\Psi_m$ decrease to 41±5% and 7±2%, respectively. Depolarized mitochondria were associated with donut-formation in 5 mM KCN, and with massive swelling in 10 mM KCN. Fusion activity decreased to 31% in 5 mM KCN and 13% in 10 mM KCN, respectively. Neither H nor KCN evoked cleavage of Opa1, the mitochondrial inner membrane fusion protein. Thus, both physical and chemical H induced respiratory inhibition to gradually lower $\Delta\Psi_m$ and cellular ATP level, and caused fusion inhibition that was not dependent on Opa1 cleavage. This is sharp contrast of the uncoupler-induced fusion inhibition that has been attributed to the rapid $\Delta\Psi_m$ dissipation-induced Opa1 cleavage. A consequence of PTP opening, the matrix swelling seems to be a key to donut formation since this could be evoked by both mastoparan, a potent PTP activator and by valinomycin, a potassium ionophore.

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Governing Respiration: Tubulin's C-Terminus Interaction with VDAC

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Mitochondria have long been known to localize within and move along the tubulin-microtubule network. It was shown that tubulin binds to isolated mitochondria with high-affinity and specifically associates with the mitochondrial voltage-dependent anion channel (VDAC). We found that nanomolar concentrations of dimeric tubulin vastly increase VDAC sensitivity to voltage allowing for VDAC blockage at low transmembrane potentials. Tubulin interaction with VDAC requires the presence of anionic C-terminal tails (CTT) on the intact protein. Tubulin with proteolytically cleaved CTTs does not block the channel. Actin, also an acidic protein but lacking CTTs, does not induce VDAC blockage. Two synthetic peptides with the sequences of mammalian α and β brain tubulin CTT do not induce detectable channel closure up to micromolar concentrations. These results suggest a completely new role for