the C terminus of Milton is an important regulator of the mitochondria associated motors and is involved in conferring the calcium sensitivity from Miro to the motors. Milton 847-1116 is sufficient for the mitochondrial binding, whereas the 750-847 amino acids are critical for the control of calcium sensitivity of mitochondrial motility.

1966-Pos

Biophysical Properties of Mitochondria Undergoing Fusion

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Emerging evidence shows the importance of genes controlling mitochondrial fusion in physiology and their deregulation in neurodegenerative and metabolic disorders. However, apart from $\Delta\Psi_m$, the biophysical properties of the fusion-competent mitochondria remain elusive. To evaluate the conditions of contact formation and fusion, we used organelle-targeted fluorescent proteins, including photoactivatable GFP, which allow tracking of individual mitochondria. In H9c2 cells, almost every mitochondrion is aligned with microtubules that provide the primary tracks for mitochondrial movement. Our results show that ~90% of fusion events involve moving mitochondria. However, fusion occurs irrespective of mitochondrial speed. Furthermore, ~80% of fusion events involve the tip portion of the mitochondrion, whereas only ~50% of these events involve organelle side. Nocodazol, a microtubule disrupting agent that inhibits mitochondrial movements decreases the fusion frequency and changes mitochondrial fusion sites. Strikingly, 80-90% of the physical contacts between adjacent mitochondria did not result in fusion events. To evaluate whether the fusion efficacy depends on the spacing between the outer and inner mitochondrial membranes we used drugs that alter the matrix volume. Valinomycin, a K^+ ionophore that induces matrix swelling evoked a decrease in mitochondrial motility leading to fewer contacts among mitochondria but the number of fusion events was maintained, indicating an increase in fusion efficacy. This change occurred at a low valinomycin concentration (0.25nM) that did not affect $\Delta\Psi_m$ or Opa1 cleavage. Nigericin (0.5μM), a K^+/H^+ ionophore that induces shrinkage of the matrix elicited fusion inhibition and mitochondrial aggregation. Importantly, no motility inhibition or Opa1 cleavage occurred at the same time and the $\Delta\Psi_m$ was increased. These results suggest that mitochondrial fusion is facilitated by mitochondrial motility, the key determinant of the inter-mitochondrial encounter numbers and preferentially involves the front-tip of the moving organelle. In addition, fusion efficacy depends on the mitochondrial matrix volume.

1967-Pos

Mitochondrial Fusion Dynamics in Human Skeletal Muscle-Derived Cells

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Mitochondria have a fundamental role in both muscle physiology and pathology. Mitochondrial fusion and fission are important for energy metabolism, calcium homeostasis and cell death. However, the mechanisms underlying mitochondrial dynamics are poorly understood, especially in physiological models such as skeletal muscle. Here we evaluated mitochondrial fusion dynamics in human skeletal muscle cells (HUSMC). Skeletal muscle satellite cells were isolated from human muscle biopsies and were maintained and differentiated in cell culture. Mitochondrial fusion events were evaluated by confocal imaging of cells expressing mitochondria matrix targeted DsRed and matrix targeted or outer mitochondrial membrane (OMM) targeted photoactivatable-GFP. When we tagged the mitochondria in ~20% of total cellular area with photoactivated-GFP, we found those mitochondria undergoing matrix fusion with a frequency of 1.3 ± 0.1 events/min/cell (n=70). Among the fusion events, 40% led to complete fusion and only 10% was followed by separation at the apparent fusion site within 20 to 40 seconds. Both complete and transient fusion events resulted mostly from longitudinal mergers, involving end to end interaction or from mergers of adjacent mitochondria in side to side orientation. Furthermore, we found that OMM and matrix fusion are sequential and separable steps, displaying 5.8 ± 1 seconds gap (n=10). Finally, we evaluated the mitochondrial fusion dynamics in HUSMC derived from both normal and malignant hyperthermia susceptible individuals. At resting state, no significant differences were found in the number of events or in their characteristics. Thus, mitochondrial fusion commonly occurs in HUSMC, and enables mixing of both soluble and integral membrane factors. This process would help to maintain the stability of mitochondrial metabolism. The relatively low frequency of the transient fusion is probably due to the parallel organization of the cytoskeletal tracks for mitochondria and to the limited mitochondrial motility.

1968-Pos

Imaging Interorganelle Contacts and Local Calcium Dynamics at the ER-Mitochondrial Interface

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The local coupling between ER and mitochondria is essential for proper cell function. A main role of the ER-mitochondrial junctions is to provide a local calcium signaling domain that is important both for keeping energy production in line with demand and for the control of apoptotic mechanisms. So far it has not been possible to visualize the tiny ER-mitochondrial contact points in living cells or to monitor the localized $[Ca^{2+}]$ changes in the narrow space between ER and mitochondria ($[Ca^{2+}]_{ER\text{-}mt}$).

Here, we exploited rapamycin-mediated heterodimerization of FKBP12 and FRB domains of fluorescent protein constructs respectively targeted to the outer mitochondrial membrane and the ER as drug-inducible inter-organelle linkers to identify ER-mitochondrial contacts and to measure the $[Ca^{2+}]_{ER\text{-}mt}$. High-resolution fluorescence imaging and 3D reconstruction revealed rapamycin-induced clustering of the ER-targeted fluorescent linker-half to the contact areas with the mitochondria without major changes in the spatial arrangement of the ER. Essentially all mitochondria displayed contacts with the ER in both RBL-2H3 and H9c2 cells. Plasma membrane-mitochondrial contacts were less frequent with ER stacks being inserted between the two organelles. Single mitochondria display discrete patches of ER contacts as well as continuous associations. Cytoplasmic and mitochondrial matrix $[Ca^{2+}]$ showed robust ER-mitochondrial Ca^{2+} transfer with considerable heterogeneity even among adjacent mitochondria. Pericam-containing linkers revealed IP_3-induced $[Ca^{2+}]_{ER\text{-}mt}$ signals that were resistant to buffering bulk cytosolic $[Ca^{2+}]_c$ increases and exceeded 9 uM. The largest $[Ca^{2+}]_{ER\text{-}mt}$ signals did not occur at the tightest associations, indicating space requirements for the Ca^{2+} transfer machinery and functional diversity among ER-mitochondrial junctions.

These studies provide direct evidence for the existence of high Ca^{2+} microdomains between the ER and mitochondria in living cells, and open new possibilities to probe the functional importance of this specialized compartment.

1969-Pos

Dependence of ER-Mitochondria Calcium Transfer on Different IP3 Receptor Isoforms

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IP3 receptors (IP3Rs) release Ca2+ from the ER, which is locally relayed to the mitochondria to control several aspects of mitochondrial function. Recent studies have suggested that type 3 IP3Rs (IP3R3) are particularly important for mediating the Ca2+ transfer at the ER-mitochondrial interface. We set out to systematically evaluate the respective role of each IP3R isoform in chicken DT40 cell lines that express only one IP3R isoform (double-knockout, DKO1, DKO2 and DKO3) or provide a null-background (triple-knockout, TKO) for analysis of mammalian IP3Rs and their mutants.

Simultaneous imaging of cytoplasmic and mitochondrial matrix [Ca2+] ([Ca2+]c and [Ca2+]m) was performed in either permeabilized cells challenged with IP3 or in muscarinic receptor overexpressing intact cells stimulated with carbachol (Cch), an IP3-linked agonist. Saturating IP3 evoked complete discharge of ER calcium and resulted in comparable [Ca2+]m increases in each DKO. Furthermore, the Cch-induced [Ca2+]c spike was closely followed by a [Ca2+]m rise in each DKO. When TKO cells were rescued with rat IP3R1 or IP3R3, the latter mediated a larger [Ca2+]c transient but the [Ca2+]m increases were very similar for both isoforms. The relationship between the [Ca2+]c peak and the corresponding [Ca2+]m response was also very similar for IP3R1 and IP3R3. To assess the impact of the release kinetic through IP3R1 in the mitochondrial Ca2+ transfer we used two point mutants of the IP3R1, which display either enhanced inhibition by Ca2+ (D426N) or are relatively insensitive to Ca2+ inhibition (D442N). D426N showed dampened [Ca2+]m signal and [Ca2+]m vs. [Ca2+]c relationship, whereas D442N displayed a steeper [Ca2+]m vs. [Ca2+]c relationship than the wild type IP3R1. Thus, each IP3R isoform can support local ER-mitochondrial Ca2+ transfer and their competence to activate mitochondrial Ca2+ uptake depends on their deactivation kinetic.