1970-Pos

Mimicking Mitochondrial Cristae Dynamics and the Amyloid - beta (1- 42) Induced Failure of Mitochondrial Cristae. A Study Involving Model Lipid Membranes

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Number of studies showed, in the case of Alzheimer's disease, abnormalities in the oxidative metabolism of mitochondria, reduced ATP production, and, mitochondrial damage (broken *cristae*), which were related, amongst others, to the excessive presence of Amyloid-beta peptide in mitochondrial *cristae*. Surprisingly, the mechanisms relating the accumulation of Amyloid-beta in the *cristae* with the large number of mitochondria with broken *cristae* was even not evoked, never mind that it is widely recognized now that mitochondria function and morphology are coupled. In our previous work (Khalifat *et al*., 2008, Biophys J 95:4924), using giant unilamellar vesicles (GUVs) for modeling mitochondrial inner membrane, we offered some original insights into the factors that determine the dynamical tubular structures of the mitochondrial inner membrane *cristae*. Furthermore, we suggested a theoretical model (Fournier *et al*., 2009, Phys Rev Lett 102:018102) for elucidating the physical background of a particular membrane instability - membrane tubule formation, triggered by modulation of local pH. In the present work, using GUVs in a similar manner, we show directly (using video-microscopy) that Amyloid-beta might itself cause brutal rupturing of the model lipid membrane and make cristae-like morphology fail. Using large unilamellar vesicles we showed as well that the Amyloid-beta induces membrane dehydration and rise of membrane viscosity. Our hypothesis is: the failure of mitochondrial inner membrane morphology might be due to very basic and purely physical mechanism - the deterioration of mechanical (visco-elastic) properties of the lipid membrane. Thereby, the local strain created during *cristae* formation could provoke the inner membrane rupture. In other words, the Amyloid-beta (1-42) could induce the lipid bilayer incapacity to support the dynamics of shape changes underlying (and inherent to) mitochondrial inner membrane normal functioning.

1971-Pos

Mitochondria Structure and Function Matures During Mammalian Cardiac Development

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The adult heart requires a precise coupling between oxidative metabolism, excitation and contraction to provide sufficient energy for each heart beat. In contrast, the early embryo, due to the hypoxic environment in utero, generates energy mainly through anaerobic glycolysis. Although the heart is the first organ to become functional in the embryo, ensuring effective circulation and embryonic survival by the mid-embryonic period, little is known about mitochondria as the embryonic heart matures. To investigate the role of energetics and mitochondrial biogenesis during murine cardiac development, we examined mitochondrial structure and function in whole hearts and cultured myocytes harvested throughout the embryonic period. Primary culture of embryonic ventricular myocytes at embryonic day (E) 9.5 displayed less mitochondrial mass and mitochondria that were shorter in length and less organized, as they did not associate closely with the contractile apparatus and resided primarily around the nucleus and cell periphery. Compared to E9.5, E13.5 ventricular myocytes displayed greater mitochondrial mass, and mitochondria that were longer, branched, networked, and more closely associated with the contractile apparatus. Data from whole hearts using multiphoton and electron microscopy confirmed these findings. Functional measurements indicated that mitochondrial membrane potential was higher at E13.5 than at E9.5, suggesting higher mitochondrial activity at later stages of development. Taken together, these data suggest that mitochondrial biogenesis and function may be important in the differentiation of early cardiac myocytes and the maturation of the heart.

1972-Pos

Cyclophilin D Modulates Mitochondrial F_OF_1 ATP Synthase by Interacting with the Lateral Stalk of the Complex

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Blue-native gel electrophoresis purification and immunoprecipitation of F_OF_1 ATP synthase from bovine heart mitochondria revealed that cyclophilin (CyP) D associates to the complex. Treatment of intact mitochondria with the membrane-permeable bifunctional reagent dimethyl 3,3-dithiobis-propionimidate crosslinked CyPD with the lateral stalk of ATP synthase, while no interactions with F_1 sector subunits, the ATP synthase natural inhibitor protein IF1 and the ATP/ADP carrier were observed. The ATP synthase-CyPD interactions have functional consequences on enzyme catalysis, and are modulated by phosphate (increased CyPD binding and decreased enzyme activity) and cyclosporin (Cs) A (decreased CyPD binding and increased enzyme activity). Treatment of MgATP submitochondrial particles or intact mitochondria with CsA displaced CyPD from membranes, and activated both hydrolysis and synthesis of ATP sustained by the enzyme. No effect of CsA was detected in CyPD-null mitochondria, which displayed a higher specific activity of the ATP synthase than wild-type mitochondria. Modulation by CyPD binding appears to be independent of IF1, whose association to ATP synthase was not affected by CsA treatment. These findings demonstrate that CyPD association to the lateral stalk of ATP synthase modulates the activity of the complex.

1973-Pos

PINK1 Deficiency and Mitochondrial Dysfunction in Neurons and Skeletal Myocytes

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Mutations in the mitochondrial kinase PINK1 cause a recessive form of Parkinson's disease. Recent studies suggest that PINK1 is important for long term cell survival and mitochondrial function in midbrain neurons by regulating mitochondrial respiration, calcium homeostasis and oxidative stress. We used live fluorescence imaging to examine the effect of PINK1 deficiency on cell metabolism in primary midbrain neurons and skeletal myotubes. We found that basal mitochondrial membrane potential ($\Delta\psi m$) was decreased in PINK1 KO neurons (to $63.7 \pm 4.2\%$, $p<0.001$) compared to wild type (WT). In contrast, the $\Delta\psi m$ was increased by $98.7 \pm 40.5\%$ ($p<0.001$) in PINK1 KO myocytes compared to WT. Despite the difference in the level of $\Delta\psi m$, in both PINK1 KO neurons and myocytes, application of oligomycin induced mitochondrial depolarisation, suggesting that $\Delta\psi m$ is partially maintained by the hydrolysis of ATP by F_1F_0-ATPases, rather than solely by respiration. Using the luciferin/luciferase assay, we showed that the ATP level was 14.6 ± 2.3 ($p<0.05$) fold higher in the muscle compared to the midbrain, which may explain the selective vulnerability of PINK1 midbrain neurons to disease. Furthermore, the ATP level was 1.8 ± 0.1 ($p<0.05$) fold higher in PINK1 KO muscle compared to WT. We have also assessed the ATP metabolism in PINK1 KO neurons and myocytes, using an indirect measurement of ATP by Mag-fura and confirmed that both the PINK1 KO and WT myocytes exhibit more glycolytic activity than neurons. This accounts for the differences in $\Delta\psi m$ between neurons and myocytes, which ultimately contributes to alterations in calcium buffering and cell survival between different cell types. Our results demonstrate that PINK1 deficiency leads to impaired mitochondrial function not only in neurons but also in myocytes. Investigation of different responses in these tissues may lead to further understanding of PINK1 function and Parkinson's disease pathogenesis.

1974-Pos

Thermodynamic Analysis of Protein-Membrane Interactions: The case of Octameric Mitochondrial Creatine Kinase

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Mitochondrial creatine kinase (MtCK) is a key enzyme for bioenergetics, membrane topology and possibly also for general organelle morphology. X-ray structural analysis (1), EM (2) and mutational studies with SPR (3,4) revealed that the large MtCK octamers bind to and ''cross-link'' mitochondrial membranes by their two identical top or bottom faces (5). These expose expose four C-terminal basic interaction motifs that interact mainly with acidic cardiolipin (4). This interaction induces cardiolipin-rich domains in the membrane (5,6). However, earlier data point to additional hydrophobic interactions (7,8). Using SPR, we have performed a thermodynamic analysis of the MtCK binding process. Main results were: (i) Affinity of the MtCK-cardiolipin interaction increases with temperature, pointing to a participation of hydrophobic interactions. (ii) Rate constants of two MtCK binding sites identified earlier differed in temperature-dependence. (iii) Thermodynamic parameters revealed that the gain in free energy of MtCK binding mainly depends on the contribution of entropy, possibly due to charge neutralization and release of bound water. These data are consistent with a two-phase model of rapid electrostatic docking of MtCK to cardiolipin, and slower anchoring via a C-terminal hydrophobic MtCK stretch. This would reinforce MtCK membrane interaction, allow integration of this bulky enzyme into the narrow mitochondrial intermembrane space, and contribute to its functional coupling with adenine nucleotide translocator.

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1975-Pos

Differential Sensitivity of Sarcomeric and Ubiquitous Isoenzymes of Mitochondrial Creatine Kinase to Oxidative Inactivation

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Oxidative modifications of creatine kinase (CK) isoenzymes are thought to play a critical role during pathologies involving oxidative stress. Reactive oxygen and nitrogen species (ROS, RNS) not only induce enzymatic inactivation, which occurs with all CK isoenzymes, but also specific damage to the mitochondrial CK isoforms, namely interference with their oligomeric state and membrane binding capacity. Using purified recombinant proteins, cell homogenates and mitochondria isolated from rat heart and brain, we have compared the two isoforms of mitochondrial CK (sarcomeric sMtCK expressed in heart and skeletal muscle, ubiquitous uMtCK expressed in other tissues) in respect to their sensitivity to oxidative inactivation induced by the drug doxorubicin or occurring spontaneously after extraction under non-reducing condition. We confirmed that sarcomeric sMtCK shows significantly higher sensitivity to oxidation and that the loss of total CK activity in heart extracts upon storage under non-reducing condition is mainly due to the inactivation of sMtCK. We could also show that the sMtCK dimer is particularly easily inactivated and that solubilization of sMtCK from membrane (promoting dimerization) makes the protein an especially vulnerable substrate for inactivation. The differential susceptibility of the two MtCK isoenzymes has been related to some differences in their molecular structures (e.g. number and surface exposure of cysteine residues).

Cryo-Electron Microscopy & Reconstruction

1976-Pos

The *Trypanosoma Brucei* Flagellum Reveals Unique and Dynamic Structures of a Nanomachine

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The *Trypanosoma brucei* flagellum is vital for the organism's locomotion, pathogenesis and cell division. It contains a microtubular axoneme, a paraflagellar rod (PFR), and connecting proteins bridging these two structures. Our investigation by cryo-electron tomography revealed a characteristic arrangement of the axoneme internal features: the 9+2 arrangement of microtubule doublets displayed radial spoke spacing not found in other organisms. We have determined that the PFR is a quasi-crystal with a unit cell that repeats every 55 nm along the length of the axoneme. Connecting proteins are attached at 55 nm intervals (the spacing of a PFR repeat) along two of the nine doublets. During flagellar bending, the PFR unit cell axial lengths remain constant while the interaxial angles vary to accommodate the quasi-crystal's expansion and compression. RNAi silencing of one of the major PFR proteins completely abolished the assembly of the PFR, and resulted in defective cell motility. Our tomographic data of this mutated flagellum also showed that the microtubule doublets are not properly arranged within the axoneme. Thus the PFR simultaneously provides structural organization to the axoneme and the flexibility and regulation required for productive locomotion.

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1977-Pos

A Uniique Density at the Portal Vertex of HSV Virions Revealed by Asymmetric Averaging of Subtomograms

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The best characterized herpesvirus is the important human pathogen, herpes simplex virus type 1 (HSV-1). The HSV-1 virion comprises 1) an icosahedral capsid which encloses the genome, 2) a surrounding variable proteinaceous layer called the tegument and 3) an enclosing lipid envelope with glycoprotein spikes. The capsid shell has the form of a T=16 icosahedron and has been studied extensively both as an isolated capsid and as the core of the virion. Several proteins have been described as minor capsid components, prominent among which is pUL6. A dodecameric ring of pUL6 proteins forms the portal, which replaces a penton at one capsid vertex. The portal has a central channel and by analogy with tailed bacteriophage, is believed to form the route for transit of the DNA into and out of the capsid. Here we describe a cryo-ET reconstruction of the intact HSV-1 virion that identifies the portal vertex and reveals a previously unsuspected structure that spans the tegument, linking the capsid to the envelope, which we term the ''tegument cylinder''. The position and nature of this structure suggests possible roles in virus assembly or transport.

1978-Pos

Electron Tomography and Molecular Modeling Study of Chemoreceptor Organization

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The movement of bacteria in response to external stimuli represents a paradigm of broad general interest for the understanding of mechanisms underlying signal transduction across cell membranes. Bacterial chemoreceptors respond to changes in concentration of extracellular ligands by undergoing conformational changes that initiate a series of signaling events, leading ultimately to regulation of flagellar motor rotation. Atomic structures for several domains of chemoreceptors, including the periplasmic ligand-binding domain, the cytoplasmic signaling domain and the HAMP domain are available, but the molecular architectures of an intact receptor dimer, or the functionally relevant trimer-of-dimer configuration have remained elusive. Here, we have used cryo-electron tomography combined with 3D averaging to determine the *in situ* 3D structure of receptor assemblies in bacterial cells that have been engineered to overproduce only the receptor for serine chemotaxis, Tsr, and lacking all other chemotaxis receptors and signaling components. We identified two major conformations of the chemotaxis receptors. Through comparative modeling and map-constrained molecular dynamics simulations, we obtained the assembly structures of tsr organized in a two dimensional array. We show that receptors are organized in trimer-of-dimer conformations with peripasmic domain, HAMP domain, and signal traction domain transiting between conformations. It is suggested that the position of the ligand binding domain and the HAMP domain play a pivotal role in mediating signal transduction across the cell membrane.

1979-Pos

Combined Approach Towards Automatic Identification of Protein Secondary Structure Elements in Volumetric Data Sets

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Single particle cryo-electron microscopy studies, but also data from tomographic experiments, often result in volumetric 3D reconstructions of low- to intermediate resolution. Although a direct atomic interpretation is not feasible at these levels of detail, one may be able to extract structural information that describes the overall conformation of the molecular system. Especially a detection of secondary structure elements, would aid in a further description of large macromolecular complexes.

On the other hand, the limited resolution often prohibits a clear visual identification of those elements.

We therefore propose a novel algorithmic tool, which is able to annotate automatically volumetric reconstructions and determines the secondary structure elements inside the maps. Our technique is based on a multi-stage analysis: In a first step, a spatial digital path filtering technique is applied, which is able to enhance local features that may characterize helices or sheets.

In a second step those features are extracted by combining the voxel information and modeling the likelihood of the presence of a secondary structure element at the specific location. To evaluate the performance of our algorithm, we have tested it using both, synthetic and experimental maps. The results show that our software is able to successfully annotate even intermediate-resolution maps. In addition, we have combined the before-mentioned algorithmic technique with our visualization system Sculptor. Sculptor provides a user-friendly environment, which enables not only an interactive pre-processing of the volume data, but also an intuitive exploration of the results.

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