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Cardiolipin Reorganization and Phase Transition Induced by Dynamin-Related Protein 1 Facilitates Mitochondrial Membrane Fission

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Cardiolipin (CL) is a unique, dimeric phospholipid essential for mitochondrial dynamics in eukaryotic cells. Dynamin-related protein 1 (Drp1), a member of the dynamin superfamily of large GTPases, maintains the balance of mitochondrial division and fusion by rapidly catalyzing mitochondrial fission. Although recent studies have indicated a role for CL in stimulating Drp1 self-assembly as well as GTPase activity on the mitochondrial surface, the exact mechanism by which CL functions in membrane fission remains unclear. Here we use a variety of fluorescence spectroscopic and imaging approaches, together with model membranes, to demonstrate that Drp1 and CL function cooperatively in effecting membrane fission in three distinct steps: (i) Drp1's preferential association with unconstrained, fluid-phase CL molecules located at a high spatial density in the membrane bilayer, (ii) CL's reorganization in concert with Drp1 self-assembly, and (iii) CL's rapid phase transition from a lamellar, bilayer structure to an inverted hexagonal, non-bilayer configuration in the presence of Drp1 and GTP, resulting in the creation of localized membrane constrictions that are primed for fission. We propose that Drp1 thus catalyzes mitochondrial division.

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VDAC3 Forms Typical Voltage-Gated, Anion-Selective, and Tubulin-Sensitive Channels

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The Voltage Dependent Anion Channel (VDAC) forms the major pathway for metabolites across the mitochondrial outer membrane (MOM). Mammalian mitochondria have three isoforms of this protein: VDAC1, VDAC2, and VDAC3 which may play different roles in the regulation of mitochondrial functions. VDAC1 is the most studied isoform, since it is the major protein of the MOM in most cells, followed by VDAC2. Both, VDAC1 and VDAC2 reconstituted into planar membranes form voltage-dependent anion-selective channels whose conductance is modulated by dimeric tubulin. As counterpart, the poor channel-forming ability of VDAC3 together with its low proficiency in restoring normal growth in pore-less yeast led to hypothesize that this isoform does not form channels and could be just a modulator of the other two isoforms. We developed an improved protocol of purification and refolding of mouse recombinant VDAC3 that allowed us to fully characterize channels formed by VDAC3 reconstituted in planar lipid membranes and to compare its channel properties with those of VDAC1 and VDAC2. We observed the typical VDAC channel behavior of VDAC3, such as high conductive open state (3.5-4.1 nS in 1M KCl) and multiple low conductive states at potentials $\geq \pm 40$mV, selectivity for anions with $P_{Cl^-}/P_{K^+} = 1.7$, and typical voltage-gating. This suggests a structural homology of VDAC3 with the other two isoforms. Interestingly, the most distinct characteristic of VDAC3 is its significantly lower sensitivity to the blockage by tubulin, which would lead to an increased permeability of this channel to ATP and other metabolites. This might explain why the down-regulation of VDAC3 expression among the three VDAC isoforms in some cancer cells causes the most drastic decrease in their mitochondrial membrane potential, or why VDAC3 is predominantly expressed in the short-term high energy demanding cells, such as spermatozoa.

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Channeling of Mitochondrial Energy in Cardiac and Cancer Cells by the Metabolically-Dependent Outer Membrane Potential

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Mitochondria are the main source of energy in eukaryotic non-proliferating aerobic cells. In cancer cells, significant part of the energy metabolism is supported by the aerobic glycolysis. Oxidative phosphorylation metabolites cross the mitochondrial outer membrane through the voltage dependent anion channel (VDAC). The possibility of the voltage-gating functioning of VDAC under physiological conditions and proposed mechanisms of generation of the outer membrane potential (OMP) require further study. In this work, we present a computational thermodynamic analysis of a possible generation of OMP by the creatine kinase(CK)-VDAC and VDAC-hexokinase(HK) complexes, as well as by their intermembrane complexes with the adenine nucleotide translocator (ANT). The CK-VDAC and VDAC-HK complexes function as direct steady-state voltage generators, using the free energy of kinase reactions, while the ANT-CK-VDAC and ANT-VDAC-HK complexes allow the application of a part of the sum of the corresponding "kinase voltage" and the inner membrane potential to the outer membrane. The developed computational models demonstrate a high probability of generation of the metabolically-dependent OMP with magnitudes high enough to close free VDACs. We suggest that in case of cardiac cells, the CK-VDAC-mediated channeling of mitochondrial energy, earlier postulated by Saks and Wallimann, leads to the metabolically-dependent generation of a high OMP during systole, thus causing electrical closure of free VDACs and essentially avoiding the mitochondrial ATP usage by non-contraction system consumers. In case of cancer cells, that have a very high percentage of hexokinase attached to mitochondria, the electrical closure of free VDACs by OMP allows the preferential VDAC-HK-mediated channeling of the mitochondrial ATP to initiate the aerobic glycolysis by an anti-turbo mode. (Research grants #111852128625 and #520154531565, Colciencias).

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Alpha-Synuclein Blocks VDAC Suggesting Mechanism of Mitochondrial Regulation and Toxicity in Parkinson Disease

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Alpha-synuclein (α-syn), an intrinsically disordered neuronal protein, is implicated in the etiology of Parkinson disease (PD) and a number of other neurodegenerative dementias. Though recent research demonstrates the involvement of α-syn in a variety of mitochondrial dysfunction in neurodegeneration, the molecular mechanism of α-syn toxicity and its effect on neuronal mitochondria remain vague. We demonstrate a functional interaction between α-syn and the voltage-dependent anion channel (VDAC), the major conduit for ATP and other bioenergetics metabolites in the mitochondrial outer membrane. We found that at nanomolar concentrations, the full-length α-syn, its 45 amino acid C-terminal truncated mutant, as well as β- and α-synuclein isoforms induce reversible and highly voltage-dependent blockage of VDAC reconstituted into planar lipid bilayers. Binding parameters varied with each isoform, revealing the key role of the negatively charged C-terminal of synucleins in blocking a positively charged pore of VDAC. Synuclein-blocked states of VDAC differ in ionic selectivity, implying the existence of several conformation states of synuclein molecules in the channel pore. We propose a model of α-syn interaction with VDAC, in which the negatively charged C-terminus of α-syn enters the net-positive channel pore, providing a steric block for ATP flux. Experiments with a yeast strain deficient in VDAC1 demonstrate that α-syn toxicity in yeast depends on VDAC, revealing α-syn interaction with the channel in living cells. Thus, our findings show the long-sought physiological and pathophysiological roles for monomeric α-syn, which reconcile previous observations of various synuclein effects on mitochondrial bioenergetics.

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Mitochondrial DNA: The Heart of the Matter

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Cardiac conduction defects and cardiomyopathy are commonly reported in patients with inborn errors of metabolism harboring pathogenic mutations in either mitochondrial or nuclear DNA (mtDNA and nDNA, respectively). We hypothesized that the pathophysiology of inborn errors of metabolism varies due to synergistic heterozygosity between the two genomes. We examined a 13-generation Mennonite pedigree with autosomal recessive cardiomyopathy due a mutation in the adenine nucleotide translocator-1 (ANT1). Substantial variability in the progression of heart disease segregated with maternal lineage, and the severity of cardiomyopathy correlated with the mtDNA haplogroups (Strauss, et al 2013). To determine the causative nature of this correlation, we examined the influence of inherited mtDNA mutations on ANT1-cardiomyopathy in the mouse. We introduced homoplasmic mtDNA ND6 or COI missense mutations into the mouse female germ line, generating mice with complex I or IV deficiency, respectively, and analyzed Ant1-dependent cardiomyopathy on the different mtDNA backgrounds. On wt mtDNA background, the Ant1-/- mice developed a distinctive concentric dilated cardiomyopathy, characterized by substantial myocardial hypertrophy and ventricular dilation. Both COI and ND6 mtDNA mutations accelerated Ant1-/- age-dependent cardiomyopathy, as evidenced by ultrastructrural abnormalities, bioenergetic defects, mtROS production, sensitized mitochondrial permeability transition, increased mtDNA damage, and heart failure, which ultimately