Mitochondria in Cell Life and Death II

2981-Pos Board B673
Investigating Tom40 Structure and Function Relationship using Single Channel Analysis
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Nearly all mitochondrial proteins are coded by nuclear genome and must be transported across mitochondrial membranes to their final functional locations. This translocation of partially folded proteins is accomplished by several multi-protein complexes in the mitochondrial outer and inner membranes. Tom40 is the central pore-forming subunit of the multi-protein translocase complex in the mitochondrial outer membrane (TOM). Although much is known regarding the general organization of the TOM complex, the molecular basis of Tom40's ability to translocate both acidic pre-sequences of matrix proteins and hydrophobic membrane proteins remains unclear. Here we have functionally investigated the role of structural domains of Tom40. Fungal Tom40 is predicted to be comprised of a membrane-spanning beta-barrel domain with strongly conserved alpha-helical domains at N- and C-termini. We have refolded and purified a full-length (FL) fungal Tom40 and N-terminal truncation mutant (Tom40-319), and characterized their electrophysiological behavior in planar lipid membranes. Our results demonstrate that recombinant Tom40 forms cation-selective channels with four well-defined conductive states, thus reconciling conflicting reports in literature regarding conductance of the channel. High cation selectivity of all conducting sublevels of Tom40 suggests the presence of a constriction zone within the channel lumen. Tom40-319 has a lower selectivity for cations, suggesting that the N-terminal alpha-helix is located in the channel lumen. Both Tom40-FL and the 319 mutant interact with specific polycationic precursor peptide of the F1β subunit of ATP synthase in a concentration- and voltage-dependent manner. However, Tom40-319 has a lower apparent affinity for pF1β than FL, which also suggests that the N-terminal alpha-helix is located inside the channel lumen. Furthermore, both Tom40 FL and the 319 mutant do not show interactions with SynB2, a synthetic peptide of similar length and charge as pF1β, thus confirming Tom40 specificity for the presequence peptide.

2982-Pos Board B674
Alpha-Synuclein Induces Mitochondrial Dysfunction Leading to a Higher Susceptibility of PTP Opening
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Mitochondrial dysfunction has been implied in Parkinson's disease (PD) leading to neuronal death. Alpha-synuclein aggregations have been shown to be a pathological hallmark in PD patients. This current study investigates a possible effect of alpha-synuclein on mitochondrial health.
We have used fluorescence imaging and TMRM to measure the mitochondrial membrane potential ($\Delta\psi_m$) in mouse primary co-cultures of neurons and astrocytes and human iPS derived neurons.
Human iPS derived neurons bearing a triplication of the alpha-synuclein gene displayed a significantly lower $\Delta\psi_m$ (61.4 ± 3.9% of control). Importantly, basal $\Delta\psi_m$ in alpha-synuclein overexpressing cells was dependent on the age of cells which possibly reflects an increase in expression levels of alpha-synuclein.
Application of oligomycin to alpha-synuclein overexpressing neurons induced a mitochondrial depolarisation, suggesting that $\Delta\psi_m$ is partially maintained through the hydrolysis of ATP by F_1F_0-ATPases due to an impaired mitochondrial respiration.
The current study found also that the stimulation of TMRM-loaded human neurons with a high laser power (565 nm) produced ROS and induced a rapid drop in $\Delta\psi_m$ which can be partially rescued by a pre-incubation with an inhibitor of the permeability transition pore (PTP) cyclosporine A. The ROS-induced PTP opening appeared much faster in human alpha-synuclein overexpressing neurons when compared to control neurons. Additionally, application of the same method to primary neuronal mouse cultures demonstrated that pre-incubation of these cells with monomeric or oligomeric alpha-synuclein induced significantly faster PTP opening.
This work was supported by the MRC and Wellcome Trust.

2983-Pos Board B675
Functional, Structural, and Computational Approaches to the Molecular Mechanism of Vdac Gating
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The Voltage-Dependent Anion Channel (VDAC) controls the fluxes of ATP/ADP and other respiratory substrates across the mitochondrial outer membrane (MOM) by using its characteristic ability to switch or "gate" between high-conducting "open" and low-conducting "closed" states. Only the open state of VDAC is permeable for most of the negatively-charged mitochondrial metabolites. Therefore, unveiling the molecular mechanism by which VDAC controls transport of metabolites across the MOM would provide insightful information about the regulation of mitochondrial respiration and metabolism. The conformational transitions underlying VDAC voltage-gating are still under debate although all models agree on a crucial role of the N-terminus in this process, either by being a part of a mobile voltage sensor domain, which slides in and out of the channel lumen, or by moving independently upon gating. It was shown that crosslinking the N-terminus α-helix to the β-barrel wall of recombinant VDAC1 reconstituted into planar lipid membranes did not prevent the gating of the channel. Furthermore, the point mutation S193E resulting in the addition of an extra negative charge inside the pore did not affect significantly the classic gating behavior observed in the WT VDAC1. This is consistent with MAS NMR results showing similar secondary structures between WT and S193E VDAC1 for the presumably open state. Computational analysis of VDAC1 WT and S193E confirms the functional data and suggests that the surface charge of the β-barrel wall of VDAC1 is an important determinant of the N-terminus packing and stability. These data lead us to propose a new mechanism of VDAC gating where N-terminus remains inside the pore during gating screening the S193 residue and the β-barrel is flexible and undergoes conformational changes that cause a partial constriction that affects selectivity upon transition to the closed states.

2984-Pos Board B676
Parkinson Disease-Associated Protein α-Synuclein Blocks VDAC Providing New Insights into Mitochondrial Toxicity
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Dysfunction of mitochondrial enzyme complexes, production of reactive oxygen species, mitochondrial outer membrane (MOM) permeabilization, enhanced apoptosis, and structural alterations of mitochondria are believed to be crucial for the onset and progression of Parkinson's disease (PD). Direct participation of small, intrinsically disordered neuronal protein α-synuclein (α-syn) in the pathogenesis of PD has been well documented. However, the mechanisms of α-syn toxicity remain elusive. Here, we studied interaction of α-syn with the major channel of MOM, voltage-dependent anion channel (VDAC), reconstituted into planar lipid membranes. We found that at nanomolar concentrations α-syn reversibly blocks VDAC in a voltage-dependent manner. Negative potentials applied from the side of α-syn addition promote the reaction, thus implicating its acidic C-terminus in the channel blockage. α-syn induces two well-defined blocked states: the first state is ~ 60% and the second, deeper blocked state is ~ 17% of the open state conductance. The probability of the second blocked state dramatically increases with the voltage. We found that selectivity of the α-syn-blocked states is less anionic than that of the open state. Kinetic analysis of α-syn-induced blockage events showed that the on-rate strongly increases with the applied voltage. The blocked time distributions are described by single exponents for both blocked states, with their average values displaying biphasic voltage dependence thus suggesting that α-syn is able to translocate through VDAC. This could explain the previously reported interaction of α-syn with mitochondrial complex-I. Even more importantly, because VDAC is a major conduit for the fluxes of ATP, ADP, and other respiratory substrates across MOM, we hypothesize that this newly demonstrated regulation of VDAC by α-syn blockage could constitute a new mechanism of mitochondrial involvement in PD pathology and perhaps also in general neurodegenerative pathogenesis.