folding, thermostability, and tolerance to limited proteolysis. In addition, pull-down and tryptophan fluorescence analyses suggest that the LIM domains physically interact to and regulate membrane penetration of the PET domain. The findings reported here favor a model where the PET domain is engaged in Pk membrane insertion, whereas the LIM domains modulate this function.

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Can An Autotransporter Protein Truly Transport Itself Across A Lipid Bilayer?

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Autotransporter (AT) proteins are synthesized with an N-terminal signal sequence, which is cleaved after secretion through the inner membrane, a passenger domain (the mature protein), and a C-terminal porin domain. The porin domain in inserted in the outer membrane (OM) and the passenger domain is secreted through this pore. OM secretion does not require ATP nor a proton gradient, therefore the driving force for efficient secretion remains unknown, nor is it known what prevents premature folding of the passenger in the periplasm. There is an ongoing debate in the literature about the role, if any, of interactions between AT proteins and periplasmic chaperones and/or OM proteins like Omp85 on AT secretion. We are developing an in vitro system to test the autonomy of AT secretion, using the model AT pertactin from Bordetella pertussis. By mixing unfolded pertactin passenger+porin domains with lipid vesicles, we will test whether the purified protein is competent for secretion across a membrane. While this does not exclude the possibility of chaperone interactions and the participation of other proteins in vivo, it would show that ATs have the capacity to cross a membrane independently, strengthening the importance of β-sheet formation as a potential driving force for OM secretion. Vesicles consisting of PC, 10% PG, and 1% NBD-PC were made; the fluorophore NBD is spread equally in the inner and outer membrane of the vesicles. Vesicle formation was confirmed by fluorescence microscopy, and quenching experiments. We have successfully expressed the isolated pertactin porin domain, and refolded it. Future work will determine the insertion of passenger+porin into vesicles, and the extent of transport, if any.

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3D solution structure of the C-terminal Chromodomain of the Chloroplast Signal Recognition Particle and its interaction with cpSRP 54 peptide

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Chloroplasts use chloroplast signal recognition particle (cpSRP) pathway to import important cargo like light harvesting chlorophyll protein (LHCP). cpSRP is unique among SRPs in being devoid of RNA. cpSRP consists of an evolutionarily conserved 54-kDa subunit (cpSRP54) and an unique 43-kDa subunit (cpSRP43). cpSRP43 subunit has four-ankyrin repeat domain at the N terminus and a C-terminal chromo domain (CD). The C-terminal CD of cpSRP43 has been shown to provide interaction sites for the cpSRP54 subunit. In addition, the chromodomain in the cpSRP43 subunit is also believed to be important for the formation of the transit complex with LHCP. Also, recent work on cpSRP 43 protein has shown that it mimics the shape and charge distribution of SRP RNA, that's missing from this system. In this context, we embarked on the structural characterization of the C-terminal CD using a variety of biophysical techniques including multidimensional NMR spectroscopy. Far UV circular dichroism spectrum of CD shows that the backbone of the protein is predominantly in the helical conformation. 1H-15N HSQC spectrum of CD is well- dispersed suggesting that the protein is structured. Complete resonance assignments (1H, 15N and 13C) in CD have been accomplished using a variety of triple resonance experiments. Chemical shift index plots show that CD is an alpha + beta protein. A detailed analysis of the three-dimensional solution structure of CD and its interaction with the cpSRP 54 peptide will be presented. The three-dimensional solution structure of CD provides valuable insights into the molecular mechanism underlying the post-translational transport and integration of LHCP on the thylakoid membrane.

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Functional Properties of Slow Skeletal Troponin T Isoforms in Cardiac Muscle Regulation

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Since human slow skeletal troponin T (HSSTnT) mRNA expression has been shown to be upregulated in end-stage heart failure, it is important to understand its possible role in cardiac muscle regulation. At least three SSTnT isoforms have been found to exist in slow skeletal muscle: HSSTnT1 (+ Exons 5 and 12), HSSTnT2 (+5, −12), HSSTnT3 (−5, −12) and HSSTnT4 (−5, +12, only found at mRNA level). Porcine papillary skinned fibers displaced with HSSTnT1, 2 or 3 and reconstituted with HCTnI-TnC complex showed a decrease in the Ca^{2+} sensitivity of force development compared to adult human cardiac troponin T (HCTnT3). The extent of TnT displacement was analyzed by measuring the unregulated tension at pCa 8.0 after TnT treatment. The maximal recovered force was increased in fibers displaced with HSSTnT1 and 3. In contrast, HSSTnT4, showed an increase in the Ca^{2+} sensitivity of force development and no change in maximal force recovered compared to HCTnT3. Using a reconstituted system, actomyosin ATPase activity containing different HSSTnT isoforms was determined. None of the HSSTnTs were found to alter the ATPase activation in the presence of Ca^{2+}. In contrast, HSSTnT2 and 3 showed an increase in the ATPase inhibition at 0.8 μM [Tn] in the absence of Ca^{2+}. All of the HSSTnTs were less soluble than HCTnT3 at three different pHs, with the HSSTnT4 having the lowest solubility. Circular dichroism experiments performed in the presence of 1M KCl, revealed structural changes between HSSTnTs and HCTnT3. Proteolytic digestion assays are being carried out to determine the susceptibility of HSSTnTs to proteolysis. These results suggest that HSSTnT isoforms may play distinct functional roles in muscle regulation and the molecular mechanism may lie in their physical-chemical properties. Supported by NIH HL-042325 and AR-050199 and AHA 0825368E.

Membrane Protein Structure I

1716-Pos Board B560

Structure Of Gamma-secretase Reveals An Active Site Facing An Internal Membrane Cavity

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The integral membrane protein complex gamma-secretase catalyzes the final step in the production of amyloid beta-peptides involved in the pathogenesis of Alzheimer's disease. Using negative-stain single-particle electron microscopy we have determined the structure of a native-like 500kDa gamma-secretase complex comprising presenilin, nicastrin, APH-1, and PEN-2 that is fully catalytically active. Antibody labeling of the extracellular domain of nicastrin was employed to ascertain the topology of the reconstruction. Active site labeling with a gold-coupled transition state analog inhibitor demonstrates that gamma-secretase contains a single active site facing a large conical internal cavity. This cavity, surrounded by a ~35Å thick transmembrane protein wall, extends from the extracellular side of the membrane to past the membrane centre, where it narrows to finally close at the cytoplasmic side. Based on our structure we suggest a model for gamma-secretase function, in which a hydrophobic transmembrane helix substrate is hydrolyzed by catalytic aspartyl moieties at the interface of a water-accessible internal cavity away from the surrounding lipid environment.

1717-Pos Board B561

A Helix-to-Coil Transition in the Transmembrane Dimer of the Amyloid Precursor Protein is Required for Proteolysis by γ-Secretase

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Processing of amyloid precursor protein (APP) by γ-secretase is the last step in the formation of the Aβ peptides associated Alzheimer's disease. MAS solid-state NMR spectroscopy is used to establish the structural features of the transmembrane (TM) and juxtamembrane (JM) domains of APP in membrane bilayers that facilitate proteolysis. Using peptides corresponding to the APP TM and JM regions (residues 618-660), we target the glycines within the TM domain, as well as residues at the γ- and ϵ-cleavage sites. We find that GxxxG motifs involving Gly625, Gly629 and Gly633 mediate TM helix homodimerization, and that the TM helix breaks at the transition point near the ϵ-cut site. We show that the insertion of three consecutive leucines at the transition point in APP695 inhibits ϵ-cleavage leading to a low production of Aβ peptides and an accumulation of the α- and β- C-terminal fragments. The leucine insertion extends the TM domain by one helical turn, whereas an insertion of three glycines does not, demonstrating that the helix-to-coil transition is required for γ-secretase processing. Our data support a progressive cleavage mechanism for APP proteolysis that depends on the helix-to-coil transition at the TM-JM boundary and unraveling of the TM α-helix.