a GUV membrane, allowed correlation of the pore formation by BAX with the ability of BAX to oligomerize in lipid membranes. As a result, we show that BAX binds lipid membranes as a monomer and then undergoes oligomerization to form BAX pore protein-lipid complexes. FCS analysis of the populations of GUVs over a period of time showed that BAX pore complexes grow in size and increase in number with time. Analysis of the diffusion coefficients of these BAX complexes using Saffman-Delbruck theory estimates that the in-membrane hydrodynamic radius of a BAX pore complex ranges from 1 to 31 nm. Formation of BAX pore complexes in a lipid membrane is inhibited in the presence of BCL-XL (in-membrane BAX is 100% monomeric) and can be rescued by the addition of cut BID. We also show confocal 3D reconstructions of a giant BAX pore with fluorescent BAX accumulating in the edge of the pore. Lifetime of the giant BAX pore (5-10 min) together with the accumulation of BAX at the edge of the pore and the loss of surface tension in a GUV support the toroidal BAX pore model.

2399-Pos

Direct Activators BID & BIM Function Like Membrane Receptors for BAX & BCL-XL

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The BCL-2 protein family is a primary regulator of apoptosis and its interaction network converges at the pro-apoptotic BAX/BAK nexus. BAX has soluble, membrane-bound, and membrane-integrated forms that are central to the management of mitochondrial permeabilization. These states, which lead to BAX pore formation and cytochrome *c* egress, are modulated by anti-apoptotic multidomain and pro-apoptotic BH3-only proteins. Using purified recombinant BCL-2 proteins and defined liposomes, the soluble->membrane transitions and pore activity modulations have been characterized.
Direct activators cBID and BIM_S instigate BAX pore formation - a process inhibitable by BCL-X_L - and these oppositional functions are dosage-dependent. BIM_S is more efficient an activator than cBID; however, BIM_S-BAX activation is more susceptible to inhibition by BCL-X_L. Since the steps of BAX activation remain controversial, we investigated the kinetics of protein-membrane binding. BAX, cBID, BIM_S, and BCL-X_L are each capable of adsorbing to membranes, albeit with differing properties. These proteins' transitions to lipid bilayers include a rapid binding step that is reversible and distinct from a slower membrane integration step. BCL-X_L and BIM_S show a comparatively high rate of binding to membranes whereas BAX and cBID are substantially slower. The membrane-resident forms of each protein have comparably strong affinities for membranes indicating that the on-rate is most influential on their in-membrane concentrations.The difference in membrane on-rates between the direct activators potentially accounts for the disparity in their BAX activation efficacies. Intriguingly, the membrane-resident forms of cBID and BIM_S were capable of driving BAX and BCL-X_L to tight membrane affinity conformations. These activities were saturable, suggesting a protein-protein interaction rather than modulation of the bulk membrane environment. These data reveal receptor-like roles for cBID & BIM_S for soluble BCL-2 proteins during the initiation of apoptosis.

2400-Pos

Biophysical Insights into Bax Oligomerization and Membrane Insertion

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The BCL2 family of proteins tightly regulates the delicate balance between life and death. Bax, a proapoptotic member of this family, acts as the penultimate factor in the apoptotic cascade by releasing apoptogenic factors such as Cytochrome C from the mitochondrial lumen. The mechanism of mitochrondrial permeabilization by BAXis not well defined. What is known is that BAX translocates to and aggregates at the outer mitochondrial membrane before cytochrome C is released, implying the insertion of the protein occurs after the aggregation event. In this work, we have evaluated the function of the oligomerization state of BAX on the insertion of the protein into artificial membranes.

2401-Pos

Bax Pore Formation: From Activation to Oligomerization

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Many of the known apoptotic pathways involve mitochondrial membrane permeabilization, just upstream of caspase activation and cell death. This key step is controlled by the Bcl-2 family of proteins, and revolves around the action of pore-forming family member Bax, which inserts in the outer mitochondrial membrane in response to apoptotic stimuli, oligomerizes and form pores. In its soluble form, Bax is known to be monomeric and to adopt a globular α-helical structure, however, little is known about its structure (or structures) when bound to the membrane, or about the stoichiometry of its membrane oligomers. We used an in vitro system consisting of 25 nm radius liposomes prepared with a lipid composition mimicking the mitochondrial membrane, in which recombinant purified full-length Bax was inserted via activation with purified tBid. We looked at the distribution of the protein on the liposomes using both fluorescence fluctuation techniques and small-angle neutron scattering. We found that although tBid activation is necessary to set off insertion of Bax into the membrane of the liposomes, Bax auto-activation plays an important role in the formation of the membrane oligomers. We observed that part of the protein inserts in the lipid bilayer, but that a significant amount of the protein mass protrudes above the membrane. This is in contrast to predictions that all of the membrane-associated Bax states are umbrella-like, with the protein's α-helices either inserted in or arranged parallel to the membrane. Upon protein insertion we also detect a thinning of the lipid bilayer, accompanied by an increase in liposome radius, an effect reminiscent of the action of antimicrobial peptides on membranes.

2402-Pos

Conformations and Interactions of BCL-2 Family Proteins: Implications For Apoptosis

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The BCL-2 family proteins are major regulators of mitochondrion-dependent programmed cell death. They includes both pro-death and pro-life proteins, which exert their activities through physical interactions with each other with other non-homologous proteins, and with intracellular membranes. The BH3-only cytotoxic protein BID is activated by caspase-8 cleavage upon engagement of cell surface death receptors. The resulting C-terminal fragment, tBID, translocates to mitochondria, triggering the release of cytotoxic molecules and cell death. The activity of tBID is regulated by its interactions with pro-survival BCL-XL and pro-death BAX, both in the cytosol and at the mitochondrial membrane. Using NMR spectroscopy we show that full length BCL-XL is soluble and monomeric in aqueous solution. Its hydrophobic C-terminal tail, which is predicted to form a transmembrane helix in lipid membranes, folds back to interact with the hydrophobic pocket known to bind the BH3 domains of pro-apoptotic proteins. The presence of the C terminus reduces the binding affinity of BCL-XL for BH3 domains 4-fold, compared to the affinity of truncated BCL-XL. The activated pro-apoptotic protein tBID adopts an α-helical but dynamically disordered conformation in solution. However, its three-dimensional conformation is stabilized when tBID engages its BH3 domain in the BH3-binding hydrophobic groove of BCL-XL to form a stable heterodimeric complex. Studies in lipid micelles show that the proteins' conformations and interactions are dramatically different in the presence of lipids.
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2403-Pos

Membrane-Targeted Soluble Form of BAK Unfolds Like an Umbrella Upon Pore-Formation

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Mitochondrial permeabilization by the pore-forming Bcl2 (B-cell lymphoma-2) proteins such as BAX or BAK constitutes a key regulatory step in the apoptotic processes. Based on the structural similarity of these to the pore-forming bacterial proteins such as colicin and the transmembrane domain of diphtheria toxin, it has been hypothesized that BAX or BAK undergoes conformational changes upon pore formation, in which the hydrophobic helical hairpin structure found in these proteins is unwrapped and inserts into the membrane. We have developed a liposomal system that recapitulates the membrane-permeabilization by BAK through pore-formation. Using a Ni(II)-nitriloacetic acid liposomal system which can conjugate hexa-histidine tagged proteins to the surface of the simulated outer mitochondrial membrane surface, we demonstrate that nanomolar concentrations of BAK when targeted to the membrane surface can efficiently permeabilize the membrane by forming large pores. Using pairs of spin labels introduced at various positions in BAK, we measured the distances between them in the BAK protein before and after pore-formation in the Ni-NTA-liposomal system. The distance between spin labeled residues 55R1 and 146R1, which are located at the αH1-αH2 loop and at the tip of the αH5-αH6 helical hairpin loop, respectively, changes from approximately