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

15 Å to 50 Å upon pore-formation. On the other hand, the distance between residues near the top of the hairpin, i.e., 75R1 and 122R1, which are located on αH2 and at the N-terminus of αH5, respectively, changes from approximately 20 Å to 25 Å. These results suggest that upon pore formation the layers covering the αH5-αH6 helical hairpin structure in BAK open up, exposing the helical hairpin structure for membrane insertion. These results are consistent with the aforementioned hypothesis regarding the conformational changes associated with the pore-forming Bcl2 proteins upon membrane permeabilization.

2404-Pos

Amphipathic Tail-Anchoring Peptide and BH3 Peptide Induced Mitochondrial Permeabilization and Apoptosis are Mechanistically Distinct

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Pro-apoptotic Bcl-2 homology domain-3 (BH3) peptides and mimetics have been developed as cancer therapeutics. Unfortunately, their cytotoxic effects are reduced in certain cancer cells by altered expression levels of various Bcl-2 family proteins. We recently found that the amphipathic tail-anchoring peptide (ATAP) from Bfl-1, a bifunctional Bcl-2 family member, displayed strong pro-apoptotic activity by permeabilizing the mitochondrial outer membrane. In this study, we tested if the activity of ATAP requires other cellular factors and whether ATAP has an advantage over the BH3 peptides or mimetics in targeting cancer cells. We reconstituted the membrane permeabilizing activity of ATAP in liposomes and found that ATAP rapidly released fluorescent molecules of the size of cytochrome c, suggesting that ATAP membrane permeabilizing activity is independent of other protein factors. ATAP permeabilized the membrane with more efficiency and potency than tBid-activated Bax protein, and unlike Bax whose pro-apoptotic activity was significantly blocked by Bcl-2, the activity of ATAP in both liposomes and cultured cells were only marginally inhibited by Bcl-2. While the pro-apoptotic activity of BH3 peptides was largely inhibited by either overexpression of Bcl-2 or Bcl-xL or nullification of Bax and Bak in cells, the apoptotic function of ATAP was not affected by these cellular factors.
Since ATAP can specifically target to mitochondria membrane and its potent apoptotic activity is not dependent on the content of Bcl-2 family proteins, it represents a promising lead for a new class of anti-cancer drugs that can potentially overcome the intrinsic apoptosis-resistant nature of cancer cells.

2405-Pos

BCL-xL Regulates ATP Synthase and Synaptic Efficiency

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Anti-apoptotic BCL-2 family proteins such as BCL-xL play a crucial role in protecting cells from death. High levels of expression of BCL-xL are also key to the maintenance of life of certain cancer cells. Healthy adult neurons also contain high levels of BCL-xL, suggesting that BCL-xL plays a role in daily neuronal function. We have found previously that over-expression of BCL-xL in cultured neurons causes an increase in the number and size of synapses and an increase in synaptic activity, providing evidence that BCL-xL causes long term changes in synaptic efficacy and structure. We now describe that in cultured hippocampal neurons, BCL-xL overexpression enhances the availability of total cellular ATP by increasing the ATP/ADP ratio. BCL-xL specifically enhances mitochondrial ATP production even while producing a marked decrease in cellular oxygen use. Although BCL-xL is usually thought to function in the mitochondrial outer membrane, our findings suggest that it creates an increase in the efficiency of cellular energy metabolism by direct protein-protein interaction with the ATP synthase beta subunit at the inner membrane. We find that recombinant BCL-xL protein increases native brain ATP synthase enzymatic activity and that pharmacological inhibitors of BCL-xL decrease the enzymatic activity of the synthase complex. In patch clamp recordings of the isolated synthasomes, ATP seals a membrane ion leak that could decrease synthase efficiency. In contrast, BCL-xL inhibitors increase the leak. The leak is different from the oligomycin-sensitive H+ ion pathway, and is not sensitive to the membrane permeant ANT inhibitor, bongkrekic acid, or to inhibitors of MitoK_{ATP}. Our findings suggest that BCL-xL improves the efficiency of mitochondrial metabolism by helping to seal a leak in the ATP synthase complex. This may allow for increased synthesis of synaptic components during long term increases in synaptic activity.

2406-Pos

Effect of Different Lipid Compositions on Mitochondrial Outer Membrane Permeabilization Assisted by the Pro-Apoptotic Proteins tBID and BAX

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Apoptosis or programmed cell death is a conserved process that serves to remove excess, damaged or infected cells in all multi-cellular organisms. Dysregulation in apoptosis can elicit important pathological conditions such as cancer and degenerative diseases. Bcl-2 family proteins critically regulate most pathways of apoptosis at the level of mitochondria. In addition to the protein-protein interactions among the Bcl-2 family members, the interaction of Bcl-2 family members with the mitochondrial outer membrane (MOM) are also very important for the execution of apoptosis. Considerable evidence supports that the composition of OMM mediates the translocation of the pro-apoptotic activator tBID to the OMM, and the subsequent activation of the pore-forming protein Bax at MOM to induce apoptosis. We have carried out a systematic study on the effect of different lipids, such as cardiolipin, monolysocardiolipin, cholesterol, and ceramide, using an *in vitro* system of liposomes to study MOM permeabilization.

2407-Pos

Structure and Dynamics of an Apoptotic Model Membrane

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In order to address the dynamic structural changes of cell membranes during apoptosis, we have studied the effect of enzymatically generated ceramide (Cer), in equimolar mixtures of palmitoyl-oleoyl-phosphatidylcholine and egg sphingomyelin (SM). Hydrolysis of SM to Cer was achieved using the well characterized neutral sphingomyelinase from bacillus cereus. By combining high performance thin layer chromatography, synchrotron time-resolved small- and wide-angle x-ray-scattering and photon correlation spectroscopy we were able to correlate the compositional changes of the bilayers to membrane structural adaptations and modifications on the macroscopic level. We found that the hyperbolic increase of Cer levels leads to an instantaneous generation of a gel phase domain. The gel phase forms initially only in the outer membrane leaflet and explains the membrane budding observed previously (1). After about 150 min a constant Cer level of 32 mol % was reached. The membranes, however, continued to swell indicating structural rearrangements due to diffusion processes, vesicle rupture/fusion, or enzyme enclosure. We observe a monotonic growth of vesicle size initiating at about the same time in agreement with vesicle aggregation, reported previously (2). This effect can be understood qualitatively in terms of reduced membrane undulations of the gel phase bilayers. Hence, we present for the first time a structural time-line that bridges the molecular to macroscopic changes occurring during apoptosis. The biological relevance of our results are supported by a remarkable agreement with the kinetics observed in Jurkat cells (3).

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2408-Pos

The Effect of Ceramide on Model Membranes and Apoptotic Cells Determined by X-Ray Scattering, Solid State NMR, and Flow Cytometry

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Ceramides (Cer) are sphingolipids involved in the development of lung alveolar cell apoptosis (programmed death) and possibly in the clearance of apoptotic cells by alveolar macrophages. Typically, the clearance process is initiated by the binding of the phosphatidylserine (PS) receptor on the macrophage plasma membrane to PS which is externalized on the plasma membrane of the apoptotic (target) cell. We use a combination of molecular and cellular methods to determine the effect of ceramides on the ability of alveolar macrophages to engulf apoptotic cells. Engulfment experiments of labeled apoptotic Jurkat cells were performed with rat alveolar macrophages (AM) obtained via bronchoalveolar lavage. AM were treated with various ceramide species and efferocytosis was quantified by flow cytometry. Using small-angle X-ray scattering and solid state 2H NMR we determined how ceramides (C6:0, C18:1) affect the molecular organization and the physical properties of PS-containing membranes. By investigating model membranes with various Cer:PS:PC ratios and deuterated species we show how ceramides alter membrane thickness, bending rigidity, and the ordering of the lipid acyl chains. These studies can