mechanism behind the recognition of the mitochondrial membrane system by the Bax-α1 sequence.

2190-Pos Board B160

Direct Interactions Between Tbid And Bcl-xlΔXl Are Enhanced In Lipid Membranes

Ana J. Garcia-Saez[1], Jonas Ries[1], Mar Orzaez[2], Enrique Perez-Paya[2], Petra Schwille[1].
[1]TU Dresden, Dresden, Germany, [2]Instituto de Investigacion Principe Felipe, Valencia, Spain.

The proteins of the Bcl-2 family are key regulators of apoptosis, but their molecular mechanisms remains controversial. Two important aspects that center the debate involve the interaction network between the pro- and antiapoptotic family members and the role of their translocation to the mitochondrial outer membrane (MOM) during apoptosis. We have used FCCS to examine quantitatively the dynamic interactions of Bid and tBid with Bcl-xLΔCt in solution and in lipid membranes. We found that only the active form tBid binds to Bcl-xLΔCt and that the membrane strongly promotes binding between them. Importantly for drug design, a BH3 peptide from Bid disrupts the tBid/Bcl-xL complex in solution but not in lipid bilayers. Our findings convincingly suggest that the most relevant interaction between tBid and Bcl-xL happens in the membrane and reveal its significance as an additional regulatory stage for MOM permeabilization.

2191-Pos Board B161

Membrane Changes during Apoptosis: Part of the Process or Characteristics of the Corpse?

Elizabeth Gibbons, Caitlin E. Askew, Katalyn R. Griffith, Michael C. Streeter, Ashley O. Warcup, Celestine H-Y Yeung, Allan M. Judd, John D. Bell.
Brigham Young University, Provo, UT, USA.

Thapsigargin-induced apoptosis in S49 lymphoma cells causes biophysical changes in the plasma membrane. Thapsigargin (TG) is a sarco/endoplasmic reticulum Ca^{2+} ATPase inhibitor that causes depletion of intracellular calcium stores and increased cytosolic calcium levels. It is used to model endoplasmic reticulum stress-induced apoptosis. This study focuses on membrane physical changes involved in this specific apoptotic pathway and possible cellular mechanisms that could account for these alterations. The fluorescent probes merocyanine 540 (MC540), laurdan, patman, and diphenylhexatriene (DPH) were used to assess lipid spacing, order, and fluidity using fluorescence spectroscopy, two-photon excitation microscopy, and confocal microscopy. MC540 fluorescence intensity increased throughout the apoptotic process, suggesting an increase in interlipid spacing. Two-photon microscopy images with laurdan showed a transient reduction in membrane order. Alexa Fluor-labeled annexin V was used to assess phosphatidylserine exposure in the outer leaflet of the plasma membrane. Flow cytometry experiments showed a sharp increase in the population showing this flip-flop after 2.5 h incubation with TG. Susceptibility of the cells to secretory phospholipase A2 (sPLA2), a hydrolytic enzyme that can distinguish apoptotic membranes from healthy ones, was also evaluated by measuring membrane permeability to propidium iodide. Two hours after addition of TG, a small population of cells became susceptible to sPLA2, and that population increased steadily with longer incubations. All of these alterations in the plasma membrane were compared temporally with caspase activation using a fluorescently labeled caspase inhibitor, FAM-VAD-fmk. This comparison suggested that caspase activation, susceptibility to sPLA2, and decreased lipid order detected by laurdan precede PS exposure, elevated MC540 fluorescence, and eventual cellular demise. Experiments are in progress with multiple pharmacological agents to assess cause and effect relationships among these events and a possible role of ceramide.

2192-Pos Board B162

Chemotherapeutic Apoptosis: Who Assailed The Membrane, The Inducer Or The Induced?

Jennifer Nelson, Kristen Barlow, D. Olin Beck, Amanda Berbert, Kelly Damm, Nathan Eschenroder, Kristina Neeley, Mark Pruitt, Kyle Thompson, Brian Thurber, Celestine H-Y Yeung, Allan M. Judd, John D. Bell.
Brigham Young University, Provo, UT, USA.

The ability of certain chemotherapeutic agents to partition into the cell membrane raises the possibility that some of their effects on cells may involve direct disruption of normal bilayer function. Moreover, previous studies on hormone-stimulated apoptosis indicate a variety of indirect membrane changes that accompany the death process including changes in membrane fluidity and order, increases in interlipid spacing, and susceptibility to hydrolysis by secretory phospholipase A2 (sPLA2). To compare the relative roles of potential direct and indirect effects of chemotherapeutic agents on cell membrane properties, we treated S49 lymphoma cells with daunorubicin (partitions in membrane) or methotrexate (non-membrane perturbing). An additional difference between the two drugs relates to their involvement of caspase-3 in the apoptotic process; daunorubicin requires it, and methotrexate does not. Membrane properties were assessed over time after addition of the drugs by fluorescence spectroscopy and microscopy using merocyanine 540, laurdan, diphenylhexatriene, and patman. The preliminary results of these studies showed commonalities between daunorubicin and methotrexate. For example, their effects on susceptibility to sPLA2 were nearly identical. Initially, the membrane remained resistant to hydrolysis for several hours. Thereafter, a sharp increase in sPLA2 activity was observed. These results suggested that changes that render the membrane vulnerable to hydrolytic attack are controlled by biochemical processes associated with apoptosis rather than reflecting direct effects of a chemotherapeutic drug on the cell membrane. Interestingly, activation of caspase-3 appeared not to be part of those processes.

2193-Pos Board B163

Designing Single Fluorescent Protein Based Caspase Sensor For Monitoring Apoptosis In Living Cells

Shenghui Xue[1], Ning Chen[2], Yun Huang[2], Jenny J. Yang[2].
[1]Department of Chemistry, Department of Biology, Georgia State University, Atlanta, GA, USA, [2]Department of Chemistry,Georgia State University, Atlanta, GA, USA.

Intracellular apoptotic signals regulated by caspase-cascade systems are closely associated with human diseases such as cancer and neurodegenerative diseases. Monitoring the activation and inhibition of caspase 3 and other caspases with fluorescence spectrum changes in living cells is essential for further understanding these processes. Here, we report progress in the development of caspase sensors based on a single fluorescent protein. These developed sensors exhibit strong enzymatic selectivity as well as high sensitivity based on observed ratiometric fluorescence changes. Additionally, our sensors can be targeted to different subcellular locations, such as the ER and mitochondria. We have further applied these sensors to monitor caspase-dependant apoptosis in different cells. Our results indicate that different inducers and drugs have diversified effects on triggering cell death pathways.

2194-Pos Board B164

Binding of the Pro-Apoptotic protein Bid to Mitochondrial Membranes is a Two Step Process

Aisha Shamas-Din, Scott Bindner, Brian Leber, David W. Andrews, Cecile Fradin.
McMaster University, Hamilton, ON, Canada.

Apoptosis is an essential process for the development of all multi-cellular organisms, and Bcl-2 family proteins critically regulate most pathways of apoptosis at the level of mitochondria. Bid is a pro-apoptotic member of Bcl-2 family proteins that regulates the integrity of OMM (Outer Mitochondrial Membrane). Upon induction of apoptosis, Bid is cleaved into a p15 and a p7 fragment, a complex that is held together by strong non-covalent interactions. The separated p15 fragment, also known as tBid (truncated Bid) is a potent inducer of cell death, however, the mechanism of the fragments' separation and activation of Bid to tBid is unknown. The focus of this work was to develop an *in vitro* fluorescent assay system to elucidate the mechanism of activation of Bid using a recombinant liposomal system bearing physiological relevance along with isolated mitochondria. Single cysteine mutants of Bid were created, and labelled with fluorescent thiol-reactive molecules to study the individual steps of the activation of Bid. Using size exclusion chromatography, FCS (Fluorescent Correlation Spectroscopy), and FRET (Förster Resonance Energy Transfer), it was quantitatively determined that the two cleaved fragments of Bid spontaneously separate upon binding to the membrane without any additional post-translational modifications. After the initial binding to the membrane, p15 fragment undergoes a conformational change to adopt its active form. Taking together, we found that the activation of Bid is a two step process encompassing the separation of the cleaved fragments and a conformational change in the membrane.

2195-Pos Board B165

Protein BAX During Detergent Activation: Characterization by Fluorescence Correlation Spectroscopy and Fluorescence Intensity Distribution Analysis

Olena Ivashyna[1], Ana J. García-Śez[2], Jonas Ries[2], Eric Christenson[1], Petra Schwille[2], Paul H. Schlesinger[1].
[1]Washington University in St. Louis, St. Louis, MO, USA.
[2]BIOTEC, TU Dresden, Dresden, Germany.

BAX is a pro-apoptotic member of the BCL-2 protein family. During apoptosis in mammalian cells cytoplasmic BAX is activated and translocates to the outer mitochondrial membrane (OMM), where it participates in formation of an

oligomeric pore capable of cytochrome c release. The biophysical mechanism of BAX activation is controversial and several *in vitro* and *in vivo* methods of its activation are known. One of the most commonly used *in vitro* methods is activation with non-ionic detergents, such as n-octylglucoside. During BAX activation with n-octylglucoside, it has been shown that BAX forms high molecular weight complexes. These complexes are ascribed to the oligomerization of BAX prior to membrane insertion and pore formation. This is in contrast with the *in vivo* studies which suggest that in cells active BAX inserts into the OMM as a monomer and then undergoes oligomerization to form a pore. Here, we used an approach which combines three single-molecule sensitivity techniques - fluorescence correlation spectroscopy (FCS), fluorescence cross-correlation spectroscopy (FCCS) and fluorescence-intensity distribution analysis (FIDA). We used FCS to determine the apparent molecular weight of the BAX-detergent micelles. The FCCS was used to determine the presence of BAX homo-oligomers in detergent micelles, while FIDA was used to determine the oligomerization number of BAX in detergent micelles. We have tested a range of detergents: n-octylglucoside, dodecylmaltoside, Triton X-100, Tween 20, CHAPS and cholic acid. With these detergents we consistently observe that BAX is a monomer before, during and after interaction with micelles. We conclude that detergent activated BAX is a monomer and that in physiological buffer conditions BAX can assume two stable monomeric conformations: one inactive and one active. This conclusion is in agreement with the *in vivo* mechanism of BAX induction of apoptosis.

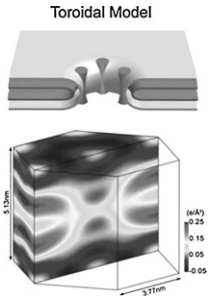
2196-Pos Board B166

Evidence for Lipidic Pores

Shuo Qian[1], Wangchen Wang[1], Lin Yang[2], Huey W. Huang[1].
[1]Rice University, Houston, TX, USA, [2]Brookhaven National Lab, Upton, NY, USA.

This study revealed the structure and mechanism of pore formation in membranes by Bax-α5 peptide, a segment from the pore-forming domain of Bax. Bax is an apoptosis regulator protein that forms pores in the outer-mitochondrial membrane to release cytochrome-c. Bax-α5 has been shown to reproduce the pore-forming activity of Bax. Bax-a5 induced pores in multiple bilayers were long-ranged correlated into a periodically ordered lattice and analyzed by X-ray anomalous diffraction. The electron density profile unambiguously shows the Bax-α5 pore is of the toroidal (wormhole) type: the two lipid monolayers merge through the pore. This was the first direct structural evidence for the existence of the long speculated lipidic pores.

The molecule mechanism of Bax-α5 pore formation was studied by two experiments: pore formation in individual GUVs exposed to Bax-α5 in solutions and the membrane thinning effect caused by the peptides. Bax-α5 exhibited a sigmoidal concentration dependence similar to antimicrobial peptides we've studied: below a threshold concentration, the peptide only binds to membrane inter surface, causing membrane thinning; when the concentration exceeds a critical value, pore formation is activated. Our results suggest that formation of such lipidic pores is a major mechanism for α-pore-forming peptides and proteins.



2197-Pos Board B167

Apoptosis Induction is Associated with VDAC Oligomerization

Varda Shoshan-Barmatz, Nurit Keinan.
Ben-Gurion University, Beer Sheva, Israel.

Mitochondria-mediated apoptosis involves efflux of a number of potential apoptotic regulators, such as cytochrome c, to the cytosol, triggering the caspase cascade and cell destruction. The precise mechanism regulating cytochrome *c* release remains unknown, and the molecular architecture of the cytochrome *c*-conducting channel has also to be determined. There is substantial evidence suggesting that the voltage-dependent anion channel-1 (VDAC1) is a critical player in apoptosis by regulating the release of apoptogenic proteins from mitochondria in mammalian cells and interacting with pro- and anti-apoptotic proteins.

Here, we demonstrate that induction of apoptosis by exposing the cells to various treatments and stimuli results in VDAC oligomerization. Staurosporine, cisplatin, curcumin, As_2O_3, etoposide, H_2O_2, UV irradiation and TNF-α, while activating mitochondria-mediated apoptosis via distinct mechanisms, all induce VDAC oligomerization (dimers to multimers). Moreover, a direct relationship between VDAC oligomerization and apoptosis, as reflected in the linear correlation between the extent of apoptosis and the level of VDAC oligomerization, was obtained. Apoptosis induction dramatically enhances VDAC1 oligomerization regardless of the cell type used, demonstrating that this phenomena is not cell-type specific. In addition, cell death induced by VDAC1 over-expression also results in highly enhanced VDAC1 oligomerization. These findings support our original proposal that oligomeric VDAC1 forms a structure which mediates the release of cytochrome *c*. We propose that VDAC1 oligomerization is a dynamic process in which apoptosis induction shifts the VDAC1 equilibrium towards oligomerization, forming a large pore allowing the release of apoptogenic proteins, such as cytochrome *c*.

2198-Pos Board B168

A Stochastic Pi-calculus Model for the Intrinsic Apoptopic Pathway

Rosaura Palma-Orozco[1], Pablo Padilla-Longoria[2], Pablo G. Padilla-Beltran[3].
[1]CINVESTAV-IPN, México D.F., Mexico, [2]IIMAS-UNAM, México D.F., Mexico, [3]ESCOM-IPN, México D.F., Mexico.

An abstract model for the intrinsic apoptopic pathway is presented. It is encoded in the stochastic pi-calculus formalism and has been tested using the SPiM simulator. The model is consistent with the current knowledge about this phenomenon. The use of this formalism allows the construction of abstract models that can be tested through virtual experiments, thus providing the ability to save resources from real experiment-based tests. Furthermore, the formalism has a proved equivalent graphical representation for describing biomolecular processes, allowing those unfamiliar with the computer science formalisms to be able to use it.

The advances in the biological science and the search of the explanations for the behavior of biological processes such as Ageing and Programmed Cell Death (PCD), make us wonder about the possibilities of finding descriptions for these processes that allow us to understand them, in order to be able to reproduce, and even control them. The need of understanding biological processes has encouraged the search for new ways to describe them, since the most common techniques (differential equations) are not suitable enough for this purpose. As result, plenty of new techniques have been developed in many areas of science, some of which are contributions from the computer science theories of processes and concurrency, the process algebras.

The main features of this calculus are the ability to describe: i) interactions and comunication between processes through the concept of name-passing; ii) structure dynamic changes in processes through mobility; and iii) stochastic behaviour by the use of a stochastic semantics. Among the advantages of this formal language for describing biological processes is the ability to test the model without actually building it physically, thus saving resources from its construction until the model has been theoretically proved to be satisfactory.

2199-Pos Board B169

Monte Carlo Simulation Shows Noisy Signaling In Apoptosis Increases Risk Of Diseases

Subhadip Raychaudhuri, Kavya Katipally.
UC Davis, davis, CA, USA.

We develop a generalized reaction class based Monte Carlo model to study signaling behavior in apoptotic cell death. We show that apoptotic signaling is noisy under weak stimuli and certain other conditions, which can explain slow apoptotic cell death as observed in recent experiments. Characteristics of such noisy signaling are large cell-to-cell stochastic fluctuations and a bimodal probability distribution for activated downstream signaling molecule caspase-3. Our study shows how genetic mutations and cell-to-cell stochastic fluctuations in apoptotic signaling can together increase risk of diseases such as cancer. Presence of a specific signaling molecule in the apoptotic pathway and its concentration are often cell type specific. This proposed Monte Carlo model is flexible so that it can be modified to include additional signaling species as well as inhibitors of the apoptotic signaling pathway. Hence, one can readily use our computational model to estimate increased risk of diseases due to faulty apoptotic signaling under various cell-type specific genetic mutations.

Protein Dynamics III

2200-Pos Board B170

A Dynamics Criterion to Determine Allostery

Ming S. Liu, Billy D. Todd, Richard S. Sadus.
Swinburne University of Technology, Hawthorn, Melbourne, Australia.

Dynamics coupling, correlated motion and allosteric cooperativity appear to be conserved in the long range communication and conformational transitions of