

mechanism behind the recognition of the mitochondrial membrane system by the Bax- $\alpha 1$ sequence.

2190-Pos Board B160

Direct Interactions Between Tbid And Bcl-xL Δ XI Are Enhanced In Lipid Membranes

Ana J. Garcia-Saez¹, Jonas Ries¹, Mar Orzaez², Enrique Perez-Paya², Petra Schwillle¹.

¹TU Dresden, Dresden, Germany, ²Instituto 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-xL Δ Ct in solution and in lipid membranes. We found that only the active form tBid binds to Bcl-xL Δ Ct 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 Ca²⁺ 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 Xue¹, Ning Chen², Yun Huang², Jenny J. Yang².

¹Department of Chemistry, Department of Biology, Georgia State University, Atlanta, GA, USA, ²Department 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 Ivashyna¹, Ana J. García-Saez², Jonas Ries², Eric Christenson¹, Petra Schwillle², Paul H. Schlesinger¹.

¹Washington University in St. Louis, St. Louis, MO, USA.

²BIOTEC, 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