Huntington's and Parkinson's. Protein misfolding is a complex phenomenon that can be facilitated, impeded, or prevented by interactions of the protein with various intracellular metabolites and intracellular nanomachines controlling protein folding. A fundamental understanding of molecular processes leading to misfolding and self-aggregation of proteins will provide critical information to help identify appropriate therapeutic routes to control these processes. Protein misfolding is the very fist link in this long chain of events eventually leading to neurodegeneration. Therefore, availability of methods capable of detecting the disease prone protein conformations facilitates the development of novel tools for diagnostic and treating the diseases at very early stages of development. This presentation summarizes our results on the development and use of the nanoimaging based approaches for detection and analysis of protein misfolding states (e.g., a review article (1)). Our approach for detecting and analyzing transient states based on the fact that misfolded conformations of a protein differ from folded and other protein conformations by their increased propensity to interact with each. We used AFM operating in the single molecule force spectroscopy mode to measure the strength of the interprotein interactions prior the aggregation. We also developed the AFM nanotweezers approach for the single molecule selection of antibodies bound to a particular type of the protein aggregates (2).

Supported by grant GM062235 (NIH) to YLL.

References

1. Lyubchenko, Y. L., Sherman, S., Shlyakhtenko, L. S. & Uversky, V. N. (2006) *J Cell Biochem* ***99***, 52–70.
2. Shlyakhtenko, L. S., Yuan, B., Emadi, S., Lyubchenko, Y. L. & Sierks, M. R. (2007) *Nanomedicine* ***3***, 192–7.

Apoptosis

2835-Pos Mitochondrial Fission During Ceramide-induced Cardiac Myocyte Apoptosis Early Steps

Veronica S. Eisner[1], Valentina Parra[1], Mario Chiong[1], Francisco Moraga[1], Alfredo Criollo[1], Alejandra Garcia[1], Steffen Härtel[1], Enrique Jaimovich[1], Antonio Zorzano[2], Cecilia Hidalgo[1], Sergio Lavandero[1]

[1] *University of Chile, Santiago Chile, Chile,*
[2] *Universitat de Barcelona, Barcelona, Spain.*

Board B138

Mitochondria are organized as a network of interconnected organelles that fluctuate between fission and fusion events (mitochondrial dynamics). The outer mitochondrial membrane protein Fis1 and the GTPase dynamin related protein-1 (Drp-1) are the main elements of the mitochondrial fission machinery. Given that 1) mitochondria fission is associated to apoptotic cell death and 2) ceramides change mitochondrial homeostasis and trigger apoptosis; we investigated here, whether activation of apoptosis with ceramides affects mitochondrial dynamics and promotes mitochondrial fission in neonatal rat cardiac myocytes primary cultures.

Cardiac myocytes mitochondrial network integrity was evaluated by 3D reconstitution of confocal microscopy images of cells loaded with mitotracker green. C2-ceramide, but not dihydro-C2-ceramide, promoted rapid fragmentation of the mitochondria network in a concentration- and time-dependent manner. C2-ceramide also increased mitochondrial Drp-1 and Fis1 content, as well as Drp-1 colocalization with Fis1, studied both, by immunofluorescence and Western blot. C2-ceramide caused a decrease in membrane potential and loss of cytochrome c mitochondria distribution pattern. To decrease the levels of the mitochondrial fusion protein mitofusin 2, we used an antisense adenovirus (AsMfn2). AsMfn2 accentuated the decrease in mitochondria membrane potential and cytochrome c redistribution induced by C2-ceramide.

We conclude that ceramides stimulate mitochondrial fission and this event is associated with early activation of cardiac myocyte apoptosis.

2836-Pos Full Length Bid, A BH3 Only Bcl-2 Family Protein, Forms A Large Pore And Transports Cytochrome C

Mitsuyoshi Saito, Curt L. Milliman, Stanley J. Korsmeyer, Paul H. Schlesinger

Washington university, St. louis, MO, USA.

Board B139

The BH3 domain only proapoptotic protein Bid plays a major role in death receptor induced mitochondria dependent apoptosis. Death receptors such as Fas/CD95 activate apical caspases that cleave full-length Bid. The C-terminal part of cleaved Bid, tBid, is believed to subsequently migrate to the mitochondria and promote the release of cytochrome c by regulating the interactions of anti-apoptotic and pro-apoptotic proteins.

In planar lipid bilayers, tBid has been shown to form a pore (1). However, the physiological importance of this pore-forming ability of tBid in cyt c release has not been examined. For that matter, the functional role of uncleaved full length Bid (flBid) in cyt c release has been even more overlooked.

Here we report that the flBid protein forms a long-lasting large conductance pore (~nano Siemens) in planar lipid bilayers and induces cyt c release from liposomes. Since flBid has been shown to bind (or transport) phospholipid (2,3), it is possible that a large conductance flBid pore is a lipid containing pore that shares its native lipidic pore properties where flBid may function to reduce the large energy requirements of lipidic pore formation.

These cyt c conducting and large pore forming abilities previously have been thought to be limited to pro-apoptotic multi-domain Bcl-2 family proteins such as Bax and Bak. This is the first evidence to show that a BH3 domain only protein, Bid, can assemble to form a large pore in lipid membranes and directly contribute to transporting cyt c.

References

1. Schendel et al. J. Biol. Chem. *274* 21932–21936 (1999).
2. Goonesinghe et al. Biochem. J. *387* 109–118 (2005).
3. Esposti et al. Mol. Cell. Biol. *21* 7268–7276 (2001).

2837-Pos Hexokinase Detachment From Mitochondria Induces Permeability Transition Pore-dependent Apoptosis

Andrea Rasola[1], Federica Chiara[1], Diego Castellaro[1], Oriano Marin[2], Valeria Petronilli[1], William SA Brusilow[3], Paolo Bernardi[1]

[1] *University of Padova Department of Biomedical Sciences, Padova, Italy*
[2] *University of Padova Department of Biological Chemistry, Padova, Italy*
[3] *Wayne State University School of Medicine, Detroit, MI, USA.*

Board B140

Hexokinase (HK) catalyses glucose phosphorylation to form glucose-6-phosphate, the first step in glucose metabolism. In highly glycolytic, neoplastic cells, the HK II isoform is over-expressed and mainly located on the outer mitochondrial membrane (OMM). HK II dissociation from mitochondria elicits cell death, making HK II a possible target for selective anti-tumour strategies. It is highly debated whether HK II partitioning away from mitochondria leads to the release of apoptogenic proteins by inducing the formation of large channels on the OMM, or by promoting the opening of the permeability transition pore (PTP) on the inner membrane.

Here we have used either the azole derivative clotrimazole or a HK II peptide to displace HK II from mitochondria. We found that these treatments efficiently detach HK II from mitochondria and trigger cell death in unrelated tumour cell models. Toxicity was abrogated by a pan-caspase inhibitor, indicating that an apoptotic pathway is initiated. Moreover, apoptosis prompted by HK II partitioning from mitochondria involved the pore regulator cyclophilin D, as it was inhibited both by cyclosporin A, a drug that targets cyclophilin D, and in cells derived from cyclophilin D knock-out mice. Pharmacological inhibition of another putative PTP regulator, the adenine nucleotide translocator, also affected the cell response to the HK II peptide and to clotrimazole. Direct measurements of Ca2+ retention capacity confirmed that the HK II peptide elicited PTP opening, without affecting neither the rate of Ca2+ uptake into the organelles nor mitochondrial respiration efficiency. Conversely, clotrimazole sensitized the pore but also inhibited respiration, making difficult to assess the specificity of its effect.

Altogether, these data indicate that the mechanism by which HK II displacement from mitochondria prompts cell death is through a PTP-dependent apoptosis, which is regulated by cyclophilin D and by the ANT.

2838-Pos Analysis Of Hydrolysis Kinetics Among Spla2 Isoforms During Apoptosis In S49 Lymphoma Cells

Erin D. Olson, Thao T. Nguyen, Allan M. Judd, John D. Bell

Brigham Young University, Provo, UT, USA.

Board B141

Secretory Phospholipase A2 (sPLA2) represents a diverse class of roughly 20 enzymes, 12 of which have been identified in humans. These isoforms can be distinguished based on their tissue distribution, function, structure, and regulation. A common feature among sPLA2 species is their ability to distinguish between healthy cells and those that are damaged through trauma or apoptosis. The mechanism by which sPLA2 distinguishes between healthy and apoptotic cells is not yet known but appears to involve changes in membrane physical properties. Previously, a relationship has been shown between the structure of the various isoforms and their propensity for hydrolyzing membranes possessing specific physical and chemical properties. Here we test the hypothesis of a relationship between enzyme structure and the ability to respond to physical changes in the membrane during apoptosis. S49 cell apoptosis was initiated by treatment with glucocorticoid (6–48 h) or with calcium ionophore. The rates of hydrolysis were compared at each treatment condition for various concentrations of snake venom and human groups (hG) IIA, V, and X isoforms. The data were analyzed using a model that explicitly evaluates both the adsorption of enzyme to the membrane surface (step 1) and subsequent binding of substrate to the active site (step 2). Increased hydrolysis during apoptosis appeared to reflect step 2 for both the snake venom and the hGX enzymes. In contrast, apoptosis promoted step 1 for hGV. For hGIIA, the kinetics were more complex suggesting additional mechanisms beyond these two steps. These observations are rationalized in terms of the structure of the various isozymes and physical changes during apoptosis including reduction in the strength of lipid/neighbor interactions and increased bilayer surface charge.

2839-Pos Relationship Between The Domain Structure And The Apoptotic Properties Of The Bcl-2 Family Proteins

Wenrui Zhao, Liying Zhou, Kang Xiao, Donald C Chang

Hong Kong University of Science and Technology, Hong Kong, China.

Board B142

The Bcl-2 family proteins contain both multi-domain anti-apoptotic (e.g., Bcl-2, Mcl-1) and pro-apoptotic (e.g., Bax, Bak) members; thus, their functions are very different. The overall structures of these two types of members, however, are very similar. To explain how the different members of the Bcl-2 family proteins may have different apoptotic properties, we investigated the structural variation in their domains. We generated a series of deletion and chimera mutants of the Bcl-2 family proteins and looked into their apoptotic property. Results of our study supported a hypothesis that the alpha 5/6 domain and the N-terminus preceding the BH3 domain together determine the apoptotic property of the protein. First, deletion of the N-terminus of the anti-apoptotic Bcl-2 family members abolished their pro-survival activity, and could even convert them into pro-apoptotic proteins, suggesting that the N-terminus is essential for the anti-apoptotic property. Second, removing the N-terminus in the pro-apoptotic Bcl-2 family members could further enhance their pro-apoptotic property, again indicating that the N-terminus is anti-apoptotic. Third, in the N-terminus deletion mutants, the apoptotic potency increased with the membrane affinity of the alpha 5/6 domains. Finally, we generated N-terminus chimera mutants between different members of the Bcl-2 family proteins. We found that none of the N-terminus could convert Bax/Bak into anti-apoptotic protein. Since the alpha 5/6 domains of Bax/Bak have extremely high membrane affinity, this result suggested that the hydrophobic

property of the alpha 5/6 domain can override the anti-apoptotic property of the N-terminus. Taken together, our results suggest that the N-terminal domain and the membrane affinity of the alpha 5/6 domain are two major factors determining the apoptotic property of the Bcl-2 family proteins.

Supported by RGC of Hong Kong, HKUST6466/05M and N_HKYST616/05.

2840-Pos Mitochondria-Mediated Apoptosis: The Role of Ceramide Channels

Leah J. Siskind[1], Meenu Perera[2], Vidyaramanan Genesan[2], J. M. Hardwick[3], R. B. Hill[4], Marco Colombini[2]

[1] *Medical University of South Carolina, Charleston, SC, USA,*
[2] *University of Maryland, College Park, MD, USA,*
[3] *Johns Hopkins University School of Public Health, Baltimore, MD, USA,*
[4] *The Johns Hopkins University, Baltimore, MD, USA.*

Board B143

Mitochondria-mediated apoptosis involves the release of pro-apoptotic proteins from the inter-membrane space to the cytosol, leading to the execution phase of apoptosis. An excellent candidate for the pathway responsible for this release is a channel formed by the sphingolipid, ceramide. Early in apoptosis, mitochondrial ceramide levels rise above the mole fraction needed for the formation of protein permeable ceramide channels in the outer membrane of rat liver mitochondria. Ceramide channels in liposomes can be visualized by negative-stain electron microscopy and their size is approximately 10 nm in diameter. Dihydroceramide, the inactive precursor lacking the essential 4,5 *trans* double bond, does not induce apoptosis nor form channels. Of the sphingolipids tested, ceramide is unique in forming protein-permeable channels.

Experiments utilizing isolated mitochondria and planar phospholipid membranes demonstrate that anti-apoptotic Bcl-2 proteins, Bcl-x_L and CED-9, favor ceramide channel disassembly while pro-apoptotic Bcl-2 proteins favor its growth. This occurs in a dose-dependent manner consistent with a 1:1 complex of anti-apoptotic protein and ceramide channel. Bcl-x_L deletion mutants that eliminate its anti-apoptotic activity have no effect on ceramide channel formation or stability. Delta-N76 Bcl-x_L was shown to be pro-apoptotic and it causes the growth of ceramide channels. Oligomeric Bax favors the growth of ceramide channels at concentrations where it causes no membrane permeabilization in the absence of ceramide. Ceramide channels have the ability, opportunity, and interactions necessary to form the gateways for the protein release pathway that initiates the execution phase of mitochondria-mediated apoptosis.

Supported by NIH NS42025, NSF MCB-0641208

2841-Pos Biophysical Changes in the Plasma Membrane during Glucocorticoid-stimulated Apoptosis Promote Hydrolysis by Secretory Phospholipase A2

Rachel W. Bailey, Elizabeth Gibbons, Leslie Robertson, Thao T. Nguyen, Jennifer Nelson, Allan M. Judd, John D. Bell

Brigham Young University, Provo, UT, USA.

Board B144

Treatment with glucocorticoid stimulates apoptosis in S49 lymphoma cells. Early during the apoptotic process, the cell membrane becomes susceptible to hydrolysis by secretory phospholipase A2 (sPLA2). Presumably, this induction of susceptibility requires specific changes in membrane physical properties. The purpose of this study was to identify the nature of those changes by examining the timing of their onset relative to increases in sPLA2 activity. Membrane properties were assessed by confocal microscopy, two-photon excitation microscopy, flow cytometry, and fluorescence spectroscopy. The following observations corresponded temporally to the induction of membrane susceptibility: increased merocyanine fluorescence intensity, red-shift in merocyanine peak wavelength, and increases in the percentage of population with high-intensity merocyanine fluorescence; exposure of phosphatidylserine (PS) to the outer leaflet, detected by annexin labeling; increased rate of extraction of NBD-labeled lipids from the plasma membrane; and complex but predictable variations in laurdan generalized polarization. Taken together, these results suggest that there is a relationship among susceptibility, reduction of interactions between neighboring phospholipids in the plasma membrane, and PS exposure. To clarify this relationship, a second set of assays was performed using dibutyryl-cAMP as the apoptotic stimulant. This drug has been reported to induce apoptosis in S49 cells without the typical exposure of PS. Our results indicated that in cells treated with dibutyryl-cAMP, the merocyanine response and its correlation with sPLA2 susceptibility was analogous to that observed with glucocorticoid-treated samples. This suggests that the underlying mechanisms which promote sPLA2 hydrolysis of apoptotic membranes lead to alterations that may be facilitated by but do not require PS exposure. Direct regulation of the biophysical microenvironment of regions of the membrane appears to be the mode of control of susceptibility to degradation by sPLA2.

2842-Pos Hypericin And Soft Laser Radiation Effects Seen In Human T Cells

Emanuel Fertig[1], Andrei Bobocea[1], Mihaela Pislea[1], Teofila Seremet[1], Eugen Radu[2], Judit Horvath[3], Ervin Tanos[3], Laszlo Katona[1], Eva Katona[1]

[1] *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[2] *"Carol Davila" University of Medicine and Pharmacy, bucharest, Romania*
[3] *LASEUROPA, Budapest, Hungary.*

Board B145

Last decades clinical experience proved the efficiency of low power farred/near-infrared laser therapy in treatment of hypoxic, ischemic, infected wounds, and chronic inflammations, but the underlying mechanisms are yet far from being fully understood, as there are the potential anti-cancerous and anti-viral effects of the polycyclic aromatic dione hypericin. Aiming to contribute to the disclosure of cellular mechanisms involved in soft lasers and polyphenols biological effects, the present studies were undertaken to monitor short and long term changes induced in human T cells state and behavior in normal and energy/nutrient restriction caused stress conditions. Human T cells were cultured in standard conditions, in presence/absence of high micromolar concentrations of hypericin. Energy/nutrient restriction was realized by serum starvation, glucose deprivation or blockade of glycolysis/oxidative phosphorylation. We used radiations emitted by AlGaInP/GaAs lasers (680nm/25mW and 830nm/55mW), and exposed the T cell suspensions to doses and irradiation regimes of therapeutic significance (total incident doses of 2–15 μ J/cell). Selecting appropriate molecular probes (PI, Hoechst, AnnexinV-FITC, 7-AAD) cell viability, proliferation rate, cell cycle progression, and percentage of apoptotic and necrotic cells were assessed by conventional, phase contrast, fluorescence microscopy, and flow cytometry. The data obtained demonstrate significant - cell state, radiation dose, and irradiation regime dependent - low power long wavelength laser irradiation effects, as well as noteworthy hypericin influence on human T cells behavior.

Partial financial support of the Romanian Ministry of Education and Research (grant CNCSIS 924/2006 and grant CEEX 6109/2005) is gratefully acknowledged

2843-Pos The pH Dependent Lipid Binding and Lipid-Induced Conformational Changes in Bax BH3 Region Mutants

Edgar L. Glover, Chirag Thakkar, Byeong Gyu Park, Juanita C. Sharpe

Chicago State University, Chicago, IL, USA.

Board B146

Bax is one of the primary pro-apoptotic Bcl-2 family protein family members involved in *cytochrome-c* release from the mitochondria and this is the key commitment step to initiating apoptosis. Bax-mediated *cytochrome-c* release may be due to the specific interaction between Bax and the mitochondrial specific lipid cardiolipin. Cardiolipin is a negatively charged lipid that is found almost exclusively in the mitochondrial membrane, and data suggest that cardiolipin may be functionally important for Bax to permeabilize membranes. Preliminary data indicate that recombinant Bax (rBax) undergoes a unique conformational change in the presence of cardiolipin that is exacerbated at low pH. We propose that cardiolipin interacts with Bax at the Bcl-2-homology 3 (BH3) region through the formation of salt-bridges between the positively charged residues in this region. Thus, we mutated the positively charged residues in the BH3 region to alanines in order to study the effect of these mutations on lipid binding and lipid-induced conformational changes. The pH dependent ability of rBax BH3 mutants K64A, K57A/K58A and K57A/K58A/K64A to bind to SUV was measured by high-speed centrifugation. Binding of WT-rBax and rBax BH3 mutants were measured in vesicles containing 30% DOPG/69.96% DOPC, 30% Cardiolipin/69.96% DOPC and 99.96% DOPC vesicles, all of which contained 0.02% Biotin-PE and 0.02% rhodamine-PE. In order to assess the effect of these mutations on the conformation of rBax, tryptophan fluorescence was measured in SUV containing 30% DOPG/70% DOPC, 30% cardiolipin/70%DOPC and 100% DOPC as a function of pH. We will discuss the effect that the charge mutations had on the pH transition at which rBax bound to lipids as well as the effect that the reduction in the number of charges in this region had on lipid-induced and cardiolipin-induced conformational changes.

2844-Pos Activated Bax Forms Oligomers Of Different Size To Permeabilize Lipid Membranes

Dmitri Satsoura, Corrie Griffiths, Brian Leber, David W. Andrews, Cécile Fradin

McMaster University, Hamilton, ON, Canada.

Board B147

Apoptosis (or programmed cell death) is a fundamental cellular process that helps regulate cell number during development and maintain tissue homeostasis. Permeabilization of the mitochondrial outer membrane (MOM), a commitment step in the apoptotic pathway, results in the release of a number of mitochondrial proteins into the cytosol thereby initiating a cascade of reactions leading to cell dismantlement and eventual cell death. Bax, a pro-apoptotic member of the Bcl-2 family of proteins, plays a key role in the formation of large pores in MOM. During apoptosis, Bax is activated and translocates from the cytosol to the MOM, where it forms membrane pores that are large enough to release proteins. The structure and composition of these pores still remains elusive today. An *in vitro* system, consisting of large unilamellar vesicles (LUVs) and recombinant proteins was developed to address the dynamic behaviour of this protein in a membrane. To detect the recombinant Bax protein it was expressed as a fusion to the fluorescent protein EGFP. Insertion of EGFP-Bax into a membrane of a LUV was triggered by adding recombinant tBid, a pro-apoptotic member of the Bcl-2 family of proteins. Oligomerization of EGFP-Bax protein in liposome membranes was investigated by fluorescence intensity distribution analysis. Our results show that EGFP-Bax forms two different types of pores in the liposomal membranes: those that are large enough to release only small dye molecules and those that can release protein molecules. Also we make predictions as to whether insertion of EGFP-Bax into liposomal membrane follows random versus non-random mechanism.

2845-Pos Dose Dependent Effect of Organophasphate Compound on Oxidative Stress and Induction of DNA Damage

Sanvidhan G. Suke, Rafat S. Ahmed, Rahul Pathak, Tanzeel Ahmed, A K. Tripathi, B. D. Banerjee

Environmental Biochemistry and Immunology Laboratory, Department of Biochemistry, University College of Medical Sciences and G.T.B. Hospital (University of Delhi), Delhi, India.

Board B148

Phosphamidon (PM) and methyl parathion (MP) are organophosphate (OP) compounds, widely used in agriculture as pesticides. We have studied the effect of PM (0.174, 0.696 and 1.74 mg/kg bw) and MP (0.218, 0.872 and 2.18 mg/kg bw) on various oxidative stress markers at 28 days exposure following oral administration in Wistar rats. Malondialdehyde (MDA) level was estimated as an index of lipid peroxidation; reduced glutathione (GSH) content in whole blood; enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) in erythrocytes; glutathione peroxidase (GPx) and glutathione reductase (GR) were estimated in serum; total thiol (SH) group in plasma was estimated using standard protocol. The ferric reducing ability of plasma (FRAP) was determined as an indicator of total antioxidant status. DNA fragmentation in lymphocytes was observed by DNA ladder assay and 8-oxo-dGua was measured by HPLC as an index of oxidative DNA damage in lymphocytes. Results of this study revealed that OP compounds induced generation of reactive species, significantly increased lipid peroxidation and caused depletion of GSH content. Activity of antioxidant cascade related enzymes like SOD, CAT, GPx and GR were significantly decreased. Similarly, total SH group and FRAP were also significantly reduced. However, at high dose, exposure of OP compounds has increased the percentage of 8-oxo-dGua level and fragmentation of DNA in lymphocytes. Oxidative DNA damage/fragmentation and lowering of total antioxidant status indicate that OP compounds may induce oxidative stress and DNA damage/apoptosis by generation of reactive oxygen species. This is an important aspect of environmental health considering the substantial sub-chronic exposure to these OP compounds in nature.

2846-Pos ATAP Perturbs Mitochondria Membrane Permeability To Induce Apoptosis Independent Of Bcl-2 Protein Function

Jae-Kyun Ko[1], Jun Peng[2], Jialing Lin[2], Jianjie Ma[1]

[1] *UMDNJ-RWJMS, Piscataway, NJ, USA,*

[2] *The University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.*

Board B149

ATAP, an amphipathic tail-anchor peptide, is a highly active pro-apoptotic molecule derived from the carboxyl-terminal domain of Bfl-1. Our previous study showed that ATAP targets specifically to mitochondria and induces caspase-dependent apoptosis that does not require Bax and Bak activities. Mutagenesis studies revealed that ATAP contains two functional motifs that are essential for its pro-apoptotic function, one that allows targeting to mitochondria and the other providing an amphipathic property to ATAP (*J Cell Sci*, ***120***, 2912). Since ATAP-induced apoptosis involves loss of mitochondrial membrane potential and release of cytochrome *c* into cytosol, we tested directly the pore-forming activity of ATAP using *in vitro* reconstitution systems. Electrophysiological studies with lipid bilayer reconstitution showed that synthetic ATAP peptide could affect the bilayer membrane permeability to monovalent cations. A mutant ATAP peptide (mHR7) that removed the amphipathic property but maintained the mitochondria binding capacity was non-toxic to cells, and at the same time non-functional in lipid bilayer membranes. Using ATAP reconstituted into liposome membrane vesicles, we found that ATAP could form large pores allowing the release of 10 kDa molecules, whereas mHR7 was impermeable to molecules as small as 0.5 kDa. Unlike Bfl-1, the tail-anchoring domain of Bcl-2 is of non-amphipathic nature. Interestingly, mutating the Bcl-2 tail-anchor domain to resemble ATAP produced potent pore-forming activity. Taken together, our results suggest that ATAP constitutes 'a lethal sting' in the Bfl-1 tail that induces cell death by permeabilizing mitochondria via its pore-forming function.

2847-Pos Photobiomodulation Of Cadmium Cellular Effects in Stress Conditions

Andrei Bobocea[1], Emanuel Fertig[1], Mihaela Pislea[1], Teofila Seremet[1], Ion O. Doaga[1], Eugen Radu[1], Judit Horvath[2], Ervin Tanos[2], Laszlo Katona[1], Eva Katona[1]

[1] "*Carol Davila*" *University of Medicine and Pharmacy, Bucharest, Romania*

[2] *LASEUROPA, Budapest, Hungary.*

Board B150

Pursuing to contribute to the understanding of molecular and cellular mechanisms involved in the highly toxic environmental pollutant cadmium detrimental and soft lasers beneficial effects, the present studies were undertaken to monitor short and long term changes induced by these agents in human T cells state and behavior in normal and in energy/nutrient restriction caused stress conditions. Human T cells were cultured in standard conditions, in presence/absence of various concentrations of $CdCl_2$. Energy/nutrient restriction was realized by serum starvation, glucose deprivation or blockade of glycolysis/oxidative phosphorylation. We used radiations emitted by AlGaInP/GaAs lasers (680nm/25mW and 830nm/55mW), and exposed the T cell suspensions to doses and irradiation regimes of therapeutic significance (total incident doses of 2–15 μJ/cell). Selecting appropriate molecular probes (JC-1, MitoTracker Green, PI, Hoechst, AnnexinV-FITC, 7-AAD) the mitochondrial reticulum state, cell viability, proliferation rate, cell cycle progression, and percentage of apoptotic and necrotic cells were assessed by conventional, phase contrast, fluorescence microscopy, and flow cytometry. The obtained data reveal significant, laser dose, irradia-

tion regime and cell state dependent photobiomodulation of cadmium effects in human T cells.

Authors are grateful for the financial support of the Romanian Ministry of Education and Research (grant CNCSIS 924/2006 and grant CEEX 60/2005).

2848-Pos How Cells Decide Between Life And Death: Predictions From Stochastic Simulation

subhadip raychaudhuri

UC Davis, davis, CA, USA.

Board B151

Programmed cell death (apoptosis) is a complex cellular process that involves two separate intracellular signaling pathways, namely Type 1 and Type 2, and a wide range of signaling molecules. A clear mechanistic understanding of how apoptotic stimulus at the cell surface triggers the apoptotic signaling cascade and a cell fate decision is made is still elusive. Using a simple effective model of apoptotic signaling we show that how apoptotic signaling is differentially regulated as we vary the strength of the death stimulus at the cell surface. Specifically we show that under weak apoptotic stimulus the slower Type 2 pathway dominates the signaling where cell-to-cell stochastic fluctuations significantly deviate from the average (over many cells) behavior. Results from our simple effective model is further corroborated by an extensive stochastic simulation of the apoptotic signaling cascade. We also show that our main results are sensitive to only a few critical parameters and robust against other details of the signaling network.

Natively Unfolded Proteins

2850-Pos Bridging The Structure-function Gap: Using Fluorine NMR To Monitor Conformational Changes In Clc-ec1, A Chloride-proton Antiporter Of Known Structure

Shelley Elvington, Merritt Maduke

Stanford University, Stanford, CA, USA.

Board B153

Members of the CLC family of chloride-transport proteins are found in numerous organisms ranging from bacteria to humans, and defects in CLC family members lead to a variety of human diseases. High resolution X-ray crystallographic data are available for the prokaryotic CLC family member ClC-ec1. Inferences that can be drawn from this structure alone are limited, however, since it represents only a static picture of the protein. To understand how transporter movement occurs on a molecular basis, static structural data must be translated into a dynamic model.

Since the ^{19}F resonance is highly sensitive to its chemical environment, ^{19}F NMR is ideally suited for identifying moving parts within a protein of known structure. We have labeled ClC-ec1 with fluorinated tyrosine and measured ^{19}F spectra at pH 7.5 (low transporter activity) and pH 5.0 (high transporter activity). The appearance of new intensities in the NMR spectrum when the pH of the wild-type protein is shifted indicates that at least 1 of the 9 F-tyr residues is changing chemical environment. This change is unlikely to result from unfolding of the protein, since spectral changes are reversible upon return of the sample to pH 7.5, and the protein remains active in assays for chloride and proton flux.

Mutating one or more tyrosine residues to phenylalanine removes the corresponding resonance from the ^{19}F NMR spectrum. We have used this technique to identify the resonance corresponding to the chloride-coordinating residue Y445. Furthermore, we have shown that activity-associated movement is occurring in at least one additional region outside the chloride permeation pathway. Work is currently underway to identify which residues contribute to the observed spectral changes, and therefore which regions of ClC-ec1 are moving during ion transport.

2851-Pos High-throughput Characterization of Intrinsically Disordered Proteins from the Protein Structure Initiative

Derrick E. Johnson, Megan D. Sickmeier, Jingwei Meng, Tanguy Le Gall, Vladimir N. Uversky, A. Keith Dunker

Indiana University School of Medicine, Indianapolis, IN, USA.

Board B154

The identification of intrinsically disordered proteins (IDPs) among the targets that fail to form satisfactory crystal structures in the Protein Structure Initiative would be a key to reducing the costs and time for determining three-dimensional structures of proteins. To resolve this problem, several Protein Structure Initiative Centers were asked to send samples of both crystallizable proteins and proteins that failed to crystallize. Initially, the abundance of intrinsic disorder was evaluated via computational analysis using Predictors of Natural Disordered Regions (PONDR®) and the potential cleavage sites and corresponding fragments were determined. Then, the target proteins were analyzed for intrinsic disorder by their resistance to protease digestion. The rates of tryptic digests of sample proteins generously provided by several Centers were compared to those of myoglobin, apomyoglobin and alpha-casein as standards of ordered, partially disordered and completely disordered proteins, respectively. Results from these digestion experiments generally correlate with the results of disorder predictions. Further analysis will be performed utilizing MS-based peptide fingerprinting to establish cut sites along with urea titrations of ANS fluorescence to determine collapsed, disordered forms of proteins in their native states. In addition, spectroscopic analysis of the samples will be performed in the presence of acrylamide, TCE, TFE, TMAO, and PEG to test specific hypotheses concerning the sequence-structure relationships for IDPs. We envision developing a standard high-throughput methodology to quickly and efficiently identify IDPs. By adding to our knowledge of IDPs, we plan on using the data