endoribonuclease (RNase) both residing on its cytosolic portion. Upon ER stress, the N-terminal luminal domain of IRE1α becomes dimerized. The dimerization induces the juxtaposition of the kinase domains, which consequently trans-autophosphorylate and activate the RNase activity to initiate splicing of the XBP1 (X-box binding protein 1) mRNA. Activated IRE1α up-regulates several pro-apoptotic genes to contribute to ER stress-induced apoptosis. Recently, we found that palmitate, a saturated fatty acid, activates the kinase and RNase activities in human hepatocellular carcinoma (HepG2) cells. However, the molecular mechanism how palmitate regulates the enzymatic activities of IRE1α has not been identified. We first addressed whether palmitate is involved in the direct physical interaction with the IRE1α protein. Using fluorescence polarization (FP)-based binding assay, we confirmed that palmitate binds to the cytosolic domain containing the kinase and nuclease domains, but not the luminal domain of IRE1α. Molecular dynamics simulations with the cytosolic domain of IRE1α suggested several amino acid residues (i.e. R635, R722, R864) potentially interact with the palmitate molecule. Site-specific mutation experiments further confirmed that the residues play an important role on the regulation of IRE1α activities. The computationally-assisted biochemical and biophysical studies provide insights on the molecular mechanisms by which palmitate initiates ER stress through IRE1α as well as possibly on the therapeutic efficacy of kinase inhibitor/activators.

Apoptosis

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Studying the Apoptosis-Regulating Functions of Bax and Bcl-2 at the Membrane Level

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The anti-apoptotic integral membrane protein Bcl-2 belongs to the Bcl-2 protein family, which functions as a major gatekeeper in the mitochondrial apoptotic pathway. This protein has been found to be highly over-expressed in many cancer tumors and also been shown to be involved in the inherent resistance to anti-cancer drugs. Our research focuses on the Bcl-2 protein and its interplay with both membranes and the pro-apoptotic Bcl-2 family protein Bax. Bax is the counter-player of Bcl-2 and is upon activation translocated from the cytosol to the mitochondrial outer membrane where it forms pores, leading to release of cytochrome c and subsequently cell death. So far we have been working on the expression and purification of Bax and Bcl-2, and managed to isolate full-length Bax using E. coli as expression system. For Bcl-2 we have performed cell-free expression, and have succeeded in the purification of the full-length protein, solubilized with detergent1. The next step is to characterize the proteins when they are reconstituted into model membranes, using methods such as CD and solid state NMR spectroscopy. We are especially interested in how the presence of oxidized lipids influences the protein-protein and protein-membrane interactions, mimicking the apoptotic conditions triggered by reactive oxygen species. In addition, we want to study Bax and Bcl-2 in the presence of intact, functional mitochondria, thus more closely mirroring the in vivo conditions of the proteins.Our ultimate goal is to provide structural information of the membrane-mediated mechanism underlying the action of Bcl-2 as a potent inhibitor of cell death, information crucial for the development of novel anti-cancer drugs.

1. Pedersen A, Wallgren M, Karlsson B. G., Gröbner G. (2011) ''Expression and purification of full-length anti-apoptotic Bcl-2 using cell-free protein synthesis'' Protein Expr. Purif., 77, 220–223.

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Membrane Binding and Dimerization of Bax Protein are Coupled to a Series of Conformational Changes

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Proteins of the Bcl-2 family are important regulators of apoptosis, and contributors of various diseases. Pro-apoptotic Bax proteins activated by BH3-only proteins translocate from the cytosol to the mitochondria, forming oligomers to permeabilize the mitochondrial outer membrane and initiate apoptosis. The underlying molecular mechanisms remain unclear. In this study we assayed Bax interactions with each other and with a model mitochondrial membrane. The results show that dimerization of Bax proteins in cytosol facilitates the membrane binding. Interaction with a peptide containing the BH3 region of Bax enhances both events, so does point mutations in Bax. Other mutations that block specific conformational changes in Bax also block the dimerization and/or membrane binding. Our study therefore suggests that Bax interaction in cytosol, binding to membrane and conformational changes are coupled reactions that eventually lead to permeabilization of the mitochondria outer membrane, thereby the potential targets for therapeutic interference. (The work was supported by the NIH grant GM062964 and the OCAST grant HR 10-121 to J.L.)

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Characterizing Bax and Bid Binding to Liposomes and Planar Membranes with Single Molecule Resolution

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The Bcl-2 family proteins regulate the initiation of apoptosis through protein-protein interactions that are often membrane dependant. The proapoptotic protein Bax is an attractive drug target to modulate apoptosis since it plays a vital role in mitochondria outer membrane permeabilization (MOMP), the point of no return in apoptosis. Bax monomers oligomerize to form pores in the membrane leading to the release of mitochondrial contents that triggers a caspase cascade killing the cell. The size and distribution of Bax pores on the membrane is poorly understood although much work has been done using liposome systems. To address this, we established a novel mitochondria-like planar membrane system with the potential to observe individual Bax molecules and single oligomeric pores. The system was evaluated to display the properties of a biological membrane and to allow binding by full length cBid and Bax labelled with fluorescent dyes. Protein binding was directly assessed using confocal microscopy and characterized by fluorescence correlation spectroscopy (FCS), fluorescence intensity distribution analysis (FIDA) and single particle detection. The binding properties of Bax to the planar membrane was compared to its binding properties to liposomes, which were assessed separately using fluorescence fluctuation methods.

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Ca^{2+} Dynamics in Apoptosis: Real-Time Data and Mathematical Modeling

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The time-dependent behavior of Ca^{2+} in statistically significant number of live apoptotic cells in the same population is measured for the first time (along with behavior of other apoptosis markers phosphatidyl serine and caspase-3/7). The Ca^{2+} dynamics shows predominantly a single peak behavior at the early stage but also multiple peak behavior in a small fraction of the cells. These data enable for the first time mathematical modeling of the Ca^{2+} dynamics in apoptosis. Towards this end we propose a physical model motivated by the role of cytochrome c binding to the IP3R in the endoplasmic reticulum membrane thereby enhancing the probability of Ca^{2+} release as demonstrated by Boehing et al. (Nat Cell Biol, 2003, *5*, 1051). The IP3R opening probability under the influence of cytochrome c is modeled as a modification of the probability in its absence in non-apoptotic cells (Mak et al. PNAS, 1998, *95*, 15821). Coupled ordinary differential equations for the dynamics of Ca^{2+} and cytochrome c are formulated and solved for the behavior under nonapoptotic and apoptotic conditions. The results are qualitatively consistent with the overall observed dynamics of Ca^{2+}, including the appearance of multiple peak behavior for certain ranges of the parameter space. The detailed analysis of the model indicates that the binding of cytochrome c to IP3R is the main source of cytosolic Ca^{2+} elevation in apoptosis while the active pumps on the plasma membrane provide the sink for the elevated cytosolic Ca^{2+} to return near the base level. The experimentally measured Ca^{2+} dynamics exhibit a significant cell-to-cell variation inherent to the stochastic nature of the underlying biochemical processes. Such variations are manifest in the modeling in terms of the uncertainites of the initial conditions in different cells upon the intiation of the apoptotic stress.

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Role of Membrane Oxidation in Controlling the Activity of Secretory Phospholipase A_2 Toward Apoptotic Lymphoma Cells

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The membranes of healthy lymphocytes normally resist hydrolysis by extracellular phospholipase A_2 ($sPLA_2$). However, they become susceptible during the process of apoptosis. Previous experiments have demonstrated the importance of certain physical changes to the membrane during cell death such as a reduction in membrane lipid order and exposure of phosphatidylserine on the membrane surface. Nevertheless, those investigations also showed that at least one additional factor was required for rapid hydrolysis by the human group IIA $sPLA_2$ isozyme (hGIIa). This study was designed to test the possibility that oxidation of membrane lipids is the additional factor. Flow cytometry and