suggesting that the two cysteine residues are not required for apoptosis or VDAC1 oligomerization.

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6P.3 Viability of *Saccharomyces cerevisiae* cells following exposure to H_2O_2 and protective effect of minocycline depend on the presence of VDAC

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Proteins involved in apoptosis are still a matter of debate. Therefore, we decided to check the effect of the presence of VDAC (voltage dependent anion selective channel) on viability of *Saccharomyces cerevisiae* cells following their exposure to H_2O_2 that is known to induce apoptosis both in *S. cerevisiae* and in mammalian cells. Mitochondria of *S. cerevisiae* contain only one channel-forming VDAC isoform (VDAC1), which simplifies studies on the channel. Using *S. cerevisiae* mutant depleted of VDAC1 (termed here VDAC) and the isogenic wild type, we have shown that VDAC is important for protection of *S. cerevisiae* cells against H_2O_2 treatment, particularly in exponential growth phase that is known to be more affected by H_2O_2. The increased viability of H_2O_2 pretreated exponentially growing cells containing VDAC was accompanied by clear changes of the cytosol redox state and was potentiated by minocycline, an antibiotic of the tetracycline family that displays cytoprotective potency. The protective effect of minocycline also coincided with distinct changes of cytosol redox state. Thus, we conclude that the ability to change the cytosol redox state following exposure to H_2O_2 or/and minocycline appears to be an intrinsic feature of exponentially growing cells (young cells) containing VDAC. Moreover, the ability seems to be crucial for both cell viability and protective effect of minocycline.

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6P.4 Apoptosis induces VDAC oligomerization as monitored in living cells

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Mitochondria are essential for cell survival, providing sources of cellular energy, as well as lying at the heart of apoptotic regulation. Mitochondria-mediated apoptosis results in the efflux of a number of potential apoptotic regulators, such as cytochrome *c*, to the cytosol, triggering the caspases cascade and cell destruction. The precise mechanism regulating cytochrome *c* release remains unknown, and the molecular architecture of the cytochrome *c* conducting channel has yet 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 (e.g. cytochrome *c*). However, the VDAC1 pore diameter is about 3 nm, too small for protein transport. Therefore, we propose that a mega pore is created between the VDAC1 monomers, allowing cytochrome *c* release. Here, the relationship between VDAC oligomerization and apoptosis induction was examined. We demonstrate that apoptosis induction by various stimuli, acting through different mechanisms, all involving mitochondria, is accompanied by an up to 20-fold increase in VDAC oligomerization, as revealed by chemical cross-linking. In addition, VDAC1 oligomeric state was directly monitored in living cells using BRET2 (Bioluminescence Resonance Energy Transfer) in cells expressing rVDAC1-GFP2 and rVDAC1-Luciferase. The BRET2 signal, indicating VDAC1 oligomerization, shows a dramatic increase upon cell exposure to apoptotic stimuli. Conversely, the apoptosis inhibitor, DIDS, inhibits staurosporine-induced VDAC1 oligomerization and decreased the BRET2 signal and apoptosis. We propose that VDAC1 oligomerization is a key step in mitochondrial-mediated apoptosis representing a general mechanism common to numerous apoptogens acting via different initiating cascades. Targeting the VDAC oligomeric status, and hence apoptosis, offers therapeutic strategies for combating cancers and neurodegenerative diseases.

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6P.5 Flux of fluorescently labeled ATP through mitochondrial outer membrane can be regulated by hexokinase binding

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Functioning of mitochondria requires maintaining the flux of ATP, ADP, and other metabolites across the mitochondrial outer membrane which is mediated primarily by Voltage Dependent Anion Channel (VDAC). Functions of VDAC have been previously examined in a suspension of isolated mitochondria by using biochemical methods and in electrophysiological experiments with artificial phospholipid membranes. Here, we applied Fluorescence Correlation Spectroscopy (FCS) to study the regulation of the functional state of VDAC by monitoring the distribution of fluorescently labeled ATP (BODIPY-FL-ATP) in a suspension of mitochondria isolated from rat liver. The addition of non-energized mitochondria to the solution of BODIPY-FL-ATP resulted in accumulation of the dye in these organelles as manifested in the appearance of FCS signal bursts of high intensity originating from single mitochondria associated with dye molecules. Unlabelled ATP markedly suppressed the BODIPY-FL-ATP accumulation in mitochondria at micromolar concentrations. NADH and NAD^+, of which the latter was less effective, as well as Koenig's polyanion, the known VDAC inhibitor, also inhibited the BODIPY-FL-ATP accumulation. The addition of hexokinase II (HKII) isolated from rat heart also led to a decrease in the BODIPY-FL-ATP accumulation, while a 15-residue peptide corresponding to the N-terminal domain of hexokinase did not produce this action. The effect of HKII was partially reversed by the hexokinase reaction product glucose 6-phosphate. Based on these results, we surmise that the FCS-detected accumulation of BODIPY-FL-ATP in mitochondria reflects ATP influx across the mitochondrial outer membrane through VDAC. By using the newly developed approach, the hexokinase-induced inhibition of the ATP flow mediated by VDAC was revealed in isolated mitochondria under physiological conditions.

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6P.6 Cell death induction of chronic lymphocytic leukemia lymphocytes using VDAC1-based peptides: A novel therapeutic approach

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