transports metabolites across the outer mitochondrial membrane. The simple transport function is indispensable for proper mitochondria functions and, consequently for cell activity, and makes VDAC crucial for a range of cellular processes including ATP rationing, calcium homeostasis and apoptosis execution. Here, we review recent data that we obtained for *Saccharomyces cerevisiae* cells used as a model system concerning the putative role of VDAC in communication between mitochondria and the nucleus. The presence of only one channel-forming VDAC isoform in *S. cerevisiae* mitochondria, i.e. VDAC1 (termed here YVDAC), simplifies studies of the channel. YVDAC mediates the cytosol reduction/oxidation (redox) state that contributes to expression and activity levels of cellular proteins including proteins that participate in protein import into mitochondria and antioxidant enzymes. For example, the expression level of Tom40, a crucial subunit of the TOM complex, correlates with the complex involvement in metabolite transport across the outer membrane as well as with levels of superoxide anion release from mitochondria. On the other hand, the cytosol redox state is important for the regulation of levels of mRNA encoding not only Tom proteins but also other proteins that participate in protein import into mitochondria, as well as proteins that are involved in mitochondria distribution and morphology, the mitochondria/nucleus communication and antioxidant activity. Simultaneously, copper-and-zinc-containing superoxide dismutase (CuZnSOD), a fundamental defence against superoxide anion, contributes to YVDAC proper activity and expression levels. Thus, regarding the obtained data, we propose that VDAC is an important element of a protein network that control functions of mitochondria by contributing to the cytosol redox state and/or by sensing the redox state. This is in agreement with the growing number of data showing that VDAC is a dynamic regulator, or even governor, of mitochondrial functions.

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6L.4 Structure and evolution of mitochondrial outer membrane proteins

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Gram-negative bacteria are the ancestors of mitochondrial organelles. Consequently, both entities contain two surrounding lipid bilayers known as the inner and outer membranes. While protein synthesis in bacteria is accomplished in the cytoplasm mitochondria import 99% of their protein ensemble from the cytosol, however in an opposite direction. In mitochondria four protein families including Sam50, VDAC, Tom40 and Mdm10 compose the set of integral β-barrel proteins embedded within the mitochondrial outer membrane (MOM). The 16-stranded Sam50 protein forms part of the sorting and assembly machinery (SAM) and shows a clear evolutionary relationship to members of the bacterial Omp85 family. By contrast, the evolution of VDAC and Tom40, both sharing the same fold cannot be traced to any bacterial precursor. This finding is in agreement with the newly adopted function of Tom40 as central part of the TOM translocation machinery. VDAC functions are more diverse and controversially discussed. Interactions of the channel to both sides of the membrane are reported in addition to the general function as exchange pore.

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6P.1 The anti-apoptotic protein Bcl2 regulates apoptosis via interaction with the mitochondrial protein, VDAC1

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The anti-apoptotic proteins of the Bcl2 family are expressed at high levels in many types of cancer. The mechanism by which these proteins regulate apoptosis is still not fully understood**,** yet it is well-established that their activity is mediated *via* interaction with mitochondria. Accumulated findings indicate that the Bcl2 family interact with the outer mitochondrial membrane protein, VDAC (voltage-dependant anion channel), a β-barrel protein recognized as a key protein in mitochondria-mediated apoptosis. In this study, the interaction of the Bcl2 with VDAC is studied. We demonstrate that purified Bcl2 interacts with VDAC-reconstituted into a planar lipid bilayer and reduced its channel conductance. In addition, synthetic peptides corresponding to the VDAC1 N-terminal region and selected cytosolic loops bound specifically, in a concentration- and time-dependent manner, to immobilized Bcl2, as revealed by real time surface plasmon resonance (SPR) technology. Moreover, expression of the VDAC1-based peptides in cells over-expressing Bcl2 prevented its protection against staurosporine-induced release of cytochrome *c* and subsequent cell death. These results point to Bcl2 as promoting tumor cell survival through binding to VDAC1, thereby inhibiting cytochrome *c* release and apoptotic cell death. Moreover, these findings suggest that interference with the binding of Bcl2 to mitochondria by VDAC1-based peptides may correspond to a practicable modality by which to potentiate the efficacy of conventional chemotherapeutic agents.

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6P.2 VDAC1 cysteine residues: Topology and function in channel activity and apoptosis

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The voltage-dependent anion channel (VDAC) is proposed to control metabolic cross-talk between mitochondria and the cytosol, as well as apoptotic cell death. It has been suggested that apoptosis is modulated by the oxidation state of VDAC. Since cysteine residues are the major targets for oxidation/reduction, we verified whether one or both VDAC1 cysteine residues are involved in VDAC1-mediated transport or apoptosis activities. To assess the function of VDAC1 cysteines in channel activity and to probe cysteine topology with respect to facing the pore or the bilayer, we used thiol-modifying agents, namely membrane permeable *N*-ethylmaleimide (NEM), bulky, charged 5-fluorescein-maleimide (5-FM), and the cross-linking reagent, BMOE. Bilayer-reconstituted VDAC conductance was decreased by 5-FM but not by NEM, while 5-FM had no effect on NEM-labeled VDAC conductance. BMOE formed dimeric VDAC1, suggesting that one of the two VDAC1 cysteine residues is exposed and available for cross-linking. The results thus suggest that one of the VDAC1 cysteine residues faces the VDAC pore while the second is oriented toward the lipid bilayer. Mutated rat (r)VDAC1 in which the two cysteines, Cys127 and Cys232, were replaced by alanines showed channel activity like native VDAC1 and, when expressed in cells, was localized to mitochondria. hVDAC1-shRNA- or siRNA-treated cells, expressing low levels of endogenous hVDAC1 together with native or cysteine-less rVDAC1 undergo apoptosis as induced by over-expression of VDAC1 or upon treatment with the reactive oxygen species-producing agents, H_2O_2, As_2O_3 or selenite,

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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