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Abstracts

S6 VDAC

Lectures

6L.1 Characterization of human VDAC isoforms: A peculiar function for VDAC3?

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VDACs are a family of pore-forming proteins mainly located in the mitochondrial outer membrane. In mammals three isoforms exist. In this work we have compared the human VDACs transformed in a yeast strain lacking the endogenous porin. VDAC1 and 2 are able to complement the lack of porin in mitochondrial respiration and modulation of ROS. VDAC3 has a limited ability to support the mitochondrial respiration and has no influence in the control of ROS production. The over-expression of VDAC isoforms in wild type yeast strain led to a dramatic sensitivity to oxidative stress, especially for VDAC3, and a shorter lifespan in respiratory conditions. Real-time PCR comparison of the isoforms indicated that in HeLa cells VDAC1 is 10 times more abundant than VDAC2 and 100 times than VDAC3. The over-expression of any single isoform caused a 10 time increase of the transcripts of VDAC2 and VDAC3, while VDAC1 is not changed by the overexpression of the other isoforms. Models of VDAC2 and VDAC3 isoforms structure showed that they could be made of a 19-strands b-barrel and an N-terminal sequence with variable features. In this work we show for the first time a functional characterization of VDAC3 in a cellular context.

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6L.2 Structure and function of the voltage dependent anion channel

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The voltage-dependent anion channel (VDAC) is the main pathway for metabolites, small molecules and ions across the outer mitochondrial membrane. A large amount of functional data has been accumulated in over 30 years of VDAC research, but the lack of a three-dimensional structure has made an understanding of VDAC function essentially impossible. This situation has changed recently, as high-resolution structures of VDAC were determined [1–3]. Here, we elucidate functional aspects of VDAC on the background of the three-dimensional NMR structure of human VDAC-1 in LDAO micelles [1] as well as on new experiments. The main part of the VDAC polypeptide chain forms a 19-stranded beta-barrel, with only the N-terminal 25 residues not being part of the barrel architecture. The dynamic properties of this N-terminal segment were determined by solution NMR spectroscopy, providing a link to the well-known voltage gating process. Further, the VDAC binding sites for the metabolite NADH and the natural ligand cholesterol were characterized structurally. We can also link our results to the native state of VDAC. The entire outside perimeter of the barrel is hydrophobic and covered by detergent molecules, compatible with the membrane bilayer topology. The inner diameter of the VDAC-1 pore is about 25 Å, consistent with published micrographs of native and native-like preparations. NMR spectroscopy and electron microscopy studies of VDAC in lipid bilayer nanodiscs provide a new means to connect micelle-bound VDAC structurally and functionally to its native state [4].

References

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6L.3 Communication between mitochondria and nucleus: Putative role for VDAC in reduction/oxidation mechanism

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Voltage dependent anion channel (VDAC) was identified in 1976 and since that time has been extensively studied. It is well known that VDAC

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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,