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mononuclear cells (PBMCs) obtained from CLL patients, while sparing those obtained from healthy donors. Cell death-inducing competence of the peptides was well correlated with the amount of CD19/CD5 cancerous CLL PBMCs, further illustrating peptides selectivity towards cancer cells. Furthermore, these VDAC1-based peptides induce apoptosis by activating the intrinsic pathway, reflected in membrane blebbing, release of mitochondrial cytochrome c, decreased cellular ATP levels, detachment of HK, and apoptotic cell death. This study thus reveals the potential of VDAC1-based peptides as a means to overcome the chemo-resistance of CLL cancer cells. In addition, a marked over-expression not only of Bcl2 but also of VDAC1, MAVS, AIF and SMAC/Diablo was observed in PBMCs from CLL patients, in comparison to those from healthy donors. This proteins expression profile can serve as a biomarker to forecast cancer development, treatment efficacy and potentially enable early diagnosis.

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Polyhydroxybutyrate Derivative Induces Cyclosporin a Sensitive Mitochondrial Depolarization in Mammalian Cultured Cells

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Polyhydroxybutyrate is a biological polyester of 3-hydroxybutyric acid (HB) that is ubiquitously present in all organisms. In higher eukaryotes PHB is found in the length of 10 to 100 HB units and can be present in free form as well as in association with proteins and inorganic polyphosphate. Our earlier studies indicate that PHB might play a significant role in mitochondrial function through participation in calcium uniporter and Permeability Transition Pore (PTP) activities. Here we tested the ability of PHB to interact with the mitochondria and regulate their function. To do this, we synthesized a fluorescein derivative of PHB (Fluo-PHB) and evaluated its distribution and effects in intact cultured HeLa cells using laser confocal microscopy. When added to the cells, Fluo-PHB rapidly accumulated inside the mitochondria. Fluo-PHB accumulation induced a transient increase of the mitochondrial membrane potential (measured using TMRM probe) indicating stimulation of the mitochondrial function. Further accumulation of Fluo-PHB led to mitochondrial membrane depolarization. This membrane depolarization was prevented by the inhibitor of the mitochondrial PTP - Cyclosporin A. Interestingly depolarization was not accompanied by mitochondrial swelling, typical for PTP opening. Fluorescein di-butyrate (Fluo-diB), used as a control compound, was able to distribute inside the cell but did not show preferential mitochondrial localization and did not affect mitochondrial function and membrane transport. Our data suggest that mitochondria are capable to actively accumulate PHB and that this accumulation leads to significant changes in the mitochondrial membrane permeability.

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Role of Polyphosphate in Mitochondria: From Modification of Energy Metabolism to Induction of the Cell Death

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Inorganic polyphosphate (polyP) is found in all living organisms ranging from bacteria to mammals. PolyP plays multiple physiological functions, which are distinct and dependent on the type of organism and the subcellular localization of the polymer. Recently we demonstrated that polyP levels are dependent on the cell metabolism and can be changed by mitochondrial substrates and inhibitors. We propose the existence of a feedback mechanism where polyP production and cell energy metabolism regulate each other. In order to investigate this we study the effect of polyP on mitochondrial oxygen consumption. We have found that application of short polyP (14 phosphate residues) or medium polyP (70 ortophosphates) significantly increase the level of respiratory coefficient by activation of state V3 and inhibition of V4 compare to control. Importantly, both short and medium polyP significantly reduced efficiency of oxidative phosphorylation (ADP/O ratio). It has previously been reported that polyP can modify membrane permeability for ions that can be a trigger for changes in mitochondrial metabolism. Furthermore polyP has been linked to activation of the mitochondrial permeability transition pore (mPTP) in different cells that also can be activated by calcium. Long, medium, and in lower degree, short polyP increase permeability of de-energised mitochondria for Ca^{2+}. This effect was dependent on inhibitor of mPTP - cyclosporine A. We also found that long polyP (130 orthophosphates) caused cell death in primary neurons and astrocytes, while medium (70) polyP had a much smaller effect and short (14) did not cause any. Thus, polyP has a multiple action on mitochondrial function from modification of mitochondrial energy metabolism to stimulation of calcium permeability and cell death.

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Type a Bax Channels: The Highly Voltage-Gated Form of this Killer Protein

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Bax, a pro-apoptotic protein, translocates from the cytosol to the mitochondrial outer membrane (MOM) where it oligomerizes and forms channels. When reconstituted into planar phospholipid membranes, Bax forms two types of channels: Type A and Type B. The former is voltage-gated and is the focus of this presentation. Electrophysiological studies of a single Type A channel show a complex gating pattern: a trimer of conductance decrements each with distinct voltage gating. The results are interpreted in terms of a linear trimer of strongly interacting subunit channels with the middle subunit oriented in the opposite direction from the others. The closing of the first subunit occurred around +70 mV with slow kinetics and steep voltage dependence. The second subunit didn't gate until the first one closed, after which, it closed with n = 13 and V0 ~ −22 mV. Only with the second subunit closed, did the third subunit can start to gate. It closed with n = 32 and V0 ~ +25 mV. Note that all the gating events are extremely voltage-dependent probably due to the oligomeric nature of the subunit channel. The restricted gating indicates that the gating domains are restricted by the state of neighboring subunits. Whereas the first and third subunits closed at positive voltage, the second subunit closed at negative indicating an opposite orientation. Adjacent trimeric channels provide opportunities for further interaction. A higher n value (20) was observed for gating of the second subunit in multi-channel membranes. This complex functional behavior provides insights into the properties and interactions of Bax proteins in membranes. These properties likely contribute to the decision-making process leading either to the formation large Bax channels and apoptosis or small Bax channels and cell survival.
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Prokaryotic Systems

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Evolution of Antibiotic Resistance through a Multi-Peaked Adaptive Landscape

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Antibiotic resistance is an evolving threat to public health. Understanding the evolution of antibiotic resistance at the genetic level is critical to develop novel strategies to diagnose and treat antibiotic resistant infections. We recently developed an automated microbial selection device, the ''morbidostat'', which is used to study the evolution of antibiotic resistance in dynamically sustained drug selection. The morbidostat adjusts drug concentrations to maintain nearly constant inhibition of bacterial growth even as evolving bacterial populations acquire higher resistance. using the morbidostat and next generation sequencing, we identified striking features in the evolution of trimethoprim resistance in five E. coli populations evolving in parallel. We found that resistance was acquired in a stepwise manner, through multiple mutations almost exclusively restricted to the gene encoding trimethoprim's target, dihydrofolate reductase (DHFR). Multiple distinct genotypes produced very similar trimethoprim resistant phenotypes, with each highly evolved strain each containing four mutations from a set of six possibilities, that were acquired in a non-random order. Never were more than four mutations acquired, despite sustained selection for further increases in drug resistance, indicating that these genotypes were local adaptive peaks.
In order to understand how the adaptive landscape of drug resistance contained multiple peaks, all combinatorial sets of adaptive mutations in the DHFR gene (96 strains) were constructed and characterized. These measurements showed that resistance evolves through an almost maximally rugged adaptive landscape with direct and indirect trajectories leading to distinct peaks. The ruggedness was not explained by pairwise incompatibilities between mutations, instead indicating 'high-order' genetic interactions between mutations. These high-order interactions were responsible for the existence of multiple adaptive peaks. One mutation was seen to have the power to control the adaptive landscape: its presence or absence largely defined the ruggedness or smoothness of the adaptive landscape.

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Protein: Protein Interactions in Control of the Escherichia Coli Biotin Protein Ligase Functional Switch

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Although many proteins are known to undergo functional switches in response to cellular signals, there are very few cases for which the detailed mechanism of