events leading to Bax-induced cytochrome *c* release. Furthermore, the presence of Kv1.3 protein in mitochondria from various cancer cells is observed, suggesting that this channel might play a role in the apoptotic signalling not only in lymphocytes but also in other cells.

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16L.4 MIMIK: The mitochondrial inner membrane intermediate conductance K^+-selective Ca^{2+}-activated channel

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A channel often observed in patch-clamp experiments on the inner membrane of mitochondria isolated from Human Colon Tumor 116 (HCT116) cells has been identified as the intermediate conductance K^+-selective Ca^{2+}-activated channel $K_{Ca}3.1$ (MIMIK) on the basis of its biophysical and pharmacological properties. The channel can exhibit different conductance states and kinetic modes, possibly reflecting post-translational modifications. As for the other known mitochondrial K^+ pores, MIMIK represents a population of a channel also present in the plasma membrane. Its mitochondrial location has been demonstrated by electrophysiological experiments on mitoplasts expressing a mito-targeted fluorescent protein and by a biochemical approach using specific markers of mitochondrial and contaminating membranes. In a limited survey of $K_{Ca}3.1$-expressing cells MIMIK has also been found in the mitochondria of HeLa cells and of a line of mouse embryonic fibroblasts, but not in those of two other colon tumour-derived cells, Caco-2 and C-26. Its presence in mitochondria thus appears to be regulated. The channel is predicted to have a role in mitochondrial physiology: moderate increases (K_{50} is about 300 nM) in matrix Ca^{2+} will cause its activation, leading to K^+ influx and depolarization in response to a Ca^{2+} signal. We are exploring the possibility that the channel may also be involved in cellular processes such as proliferation or death.

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Posters

16P.1 Voltage-gated potassium channel in hippocampus mitochondria

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Transient cerebral ischemia is known to induce endogenous adaptive mechanisms such as the activation of mitochondrial ATP regulated or Ca^{2+} regulated large conductance potassium channels that can prevent or delay neuronal injury. However, molecular mechanism of this effect remains unclear. In this study, a single channel activity was measured with patch-clamp of the mitoplasts isolated from gerbil hippocampus. In 70% of all the patches, a potassium selective current with properties of the voltage-gated potassium channel (Kv type channel) was recorded with mean conductance 109 ± 6 pS in symmetrical 150 mM KCl solution. Detected channel was blocked by negative voltage and margatoxin (MgTx) a specific Kv1.3 channel inhibitor. The inhibition by MgTx was irreversible. We observed that ATP/Mg^{2+} complex or Ca^{2+} ions had no effects on observed activity of ion channel. Additionally, we showed that agitoxin-2 (AgTx-2), potent inhibitor of the voltage-gated potassium channels, was without effect on channel activity. This observation suggests that mitochondrial voltage-gated potassium channel can represent different molecular structures without affinity to AgTx-2 in comparison to surface membrane channels. Also, Western blot analysis of mitochondria isolated from gerbil hippocampus and immunohistochemistry on gerbil brain sections confirm the expression of Kv1.3 protein in mitochondria. All together, we conclude that gerbil hippocampal mitochondria contain voltage-gated potassium channel (mitoKv1.3 channel) with properties similar to the surface membrane Kv1.3 channel which can influence function of mitochondria in physiological and pathological conditions.

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16P.2 Molecular dynamics of the mitochondrial protein translocase TIM22: Structure–function correlations of the channel's partakers

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Mitochondrial translocases convey the precise relocation of cytoplasmic encoded proteins to one of the four discrete compartments enfolded by their two membranes. Among their multiple subunits, those forming the aqueous channels have proven essential for the functioning of TOM andTIM23 translocases. Previously we have reported the conditions to uncover *in organello* the channel activity of TIM22, the translocase mediating the insertion of multispaning proteins into the inner membrane. Only cargo proteins facing the intermembrane space trigger the activity of this otherwise silent channel. Three membrane proteins: Tim22p, Tim54p and Tim18p partake TIM22. We have performed the molecular dissection of TIM22 present in mitochondria of eight yeast strains with different expression levels of its defined components. These results combined with those of the native complex and those of patch-clamping the inner membranes of their mitochondria, outline the biogenesis of the complex and the role played by each component. Our results indicate that Tim22p is present in a complex of about 380 kDa also containing Tim18p and Tim54p. The biogenesis of this complex depends on the simultaneous presence of the three membrane proteins. Tim54p