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.

References

[1] Szabò *et al.* (2005) *J. Biol. Chem.* **280:** 12790–12798.
[2] Szabò *et al.* (2008) *Proc. Natl. Acad. Sci. USA* **105:** 14861–14866.
[3] Gulbins *et al.* (2010) *Biochim. Biophys. Acta-Bioenergetics* (in press).

doi:10.1016/j.bbabio.2010.04.394

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

Mario Zoratti[1,2], Umberto De Marchi[3], Nicola Sassi[2], Bernard Fioretti[4], Ildikò Szabò[5]
[1]*CNR Institute of Neuroscience, Padova, Italy*
[2]*University of Padova, Department of Biomedical Sciences, Italy*
[3]*University of Geneva, Department of Cellular Physiology and Metabolism, Switzerland*
[4]*University of Perugia, Department of Cellular and Environmental Biology, Italy*
[5]*University of Padova, Department of Biology, Italy*
E-mail: zoratti@bio.unipd.it

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.

doi:10.1016/j.bbabio.2010.04.395

Posters

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

Piotr Bednarczyk[1,2], Joanna E. Kowalczyk[3], Krzysztof Dołowy[2], Adam Szewczyk[1], Barbara Zabłocka[3]
[1]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland*
[2]*Warsaw University of Life Sciences - SGGW, Department of Biophysics, Warsaw, Poland*
[3]*Mossakowski Medical Research Institute, Molecular Biology Unit, Warsaw, Poland*
E-mail: p.bednarczyk@nencki.gov.pl

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.

This study was supported by the grant from the Ministry of Science and Higher Education (P-N/31/2006) and Polish Mitochondrial Network MitoNet.pl. P.B. would like to acknowledge Prof. Detlef Siemen for teaching patch-clamp and more discussion during postdoc in Magdeburg which was supported by EMBO (2006) and DAAD (2008).

doi:10.1016/j.bbabio.2010.04.396

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

Pablo Peixoto[1,2], Lauro González-Fernández[1], Patricia Rojo[1], Jorge Bermejo[1], María Luisa Campo[1]
[1]*University of Extremadura, Department of Biochemistry and Molecular Biology, Cáceres, Spain*
[2]*New York University, College of Dentistry, New York, USA*
E-mail: mlcampo@unex.es

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

seems to be the protein that holds Tim22p and Tim18p. On other hand, the channel activity of TIM22 is greatest while the complex is intact. When disassembled, the detection frequency parallels the precise levels of Tim22p and Tim18p but is independent of Tim54p. In addition, overexpression of either Tim22p or Tim18p does not correlate with an increase in channel's frequency, despite it was described that reconstituted Tim22p alone forms pores. Taken together, these results imply that Tim18p is the putative receptor for internal signal peptides that trigger the activity of the TIM22 channel, formed by Tim22p.

Supported by Spanish MCIN BFU2008-475 and Junta de Extremadura PRI07A072.

doi:10.1016/j.bbabio.2010.04.397

16P.3 Mitochondrial potassium ion channels from embryonic hippocampal neurons

Anna Kajma[1], Piotr Bednarczyk[1,2], Adam Szewczyk[1]
[1]*Nencki Institute of Experimental Biology, Department of Biochemistry, Poland*
[2]*Warsaw University of Life Sciences - SGGW, Department of Biophysics, Poland*
E-mail: a.kajma@nencki.gov.pl

Recently, it has been shown that potassium transport, *via* ion channels, through the mitochondrial inner membrane can trigger neuroprotection. Until now five different mitochondrial potassium channels have been reported: ATP-regulated (mitoK_{ATP}), large-conductance calcium activated (mitoBK_{Ca}), intermediate conductance calcium-activated ($K_{Ca}3.1$), voltage-dependent (mitoKv1.3) and two-pore domain potassium channel (TASK-3). Our data provides evidence for the presence of mitoBK_{Ca} channels in the inner mitochondrial membrane of the rat hippocampus. The channel conductance calculated based on current-voltage relations was equal to 289 pS. The activity of the channel decreased at the low calcium concentration. The effect was reversed after application of NS1619, an activator of the BK type channels. Additionally, channel activity was blocked by paxilline (inhibitor of the BK type channels). Moreover we demonstrated that the probability of opening this channel is increased after the application of the arachidonic acid. We also identified a novel channel by patch-clamp, which has current–voltage characteristics similar to the rectifying channels. The channel conductance was equal to 60 pS. Patch-clamp studies showed that this channel is not sensitive to the known activators and inhibitors of the mitochondrial potassium channels. This channel was sensitive to the changes in the intracellular pH, and also regulated by the arachidonic acid.

Grant sponsor: Ministry of Science and Higher Education P-N/031/2006.

doi:10.1016/j.bbabio.2010.04.398

16P.4 Potassium and chloride channel activities from potato *Solanum tuberosum* tuber mitochondria

Izabela Koszela-Piotrowska[1], Karolina Matkovic[2], Wieslawa Jarmuszkiewicz[2], Adam Szewczyk[1]
[1]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland*
[2]*Laboratory of Bioenergetics, Adam Mickiewicz University, Poznan, Poland*
E-mail: i.piotrowska@nencki.gov.pl

Single channel activities were measured after reconstitution of the inner membranes from potato tuber mitochondria into planar lipid bilayers. After incorporation, in gradient 50/450 mM KCl (*cis*/*trans*), we found the ATP-regulated potassium channel with mean conductance of 164 pS, which the channel activity was inhibited by 1 mM Mg/ATP. Another detected one was the large-conductance potassium channel, which revealed the characteristic features for mammalian BK_{Ca} channels, i.e. the channel conductance was equal to 312 pS and was blocked by 200–400 nM iberiotoxin (IbTx) but did not exert the sensitivity on calcium. Furthermore, we observed also the chloride channel activity that was inhibited by 200 μM DIDS. The mean channel conductance of 117 pS and kinetic behavior were similar to that of those characterized for mitochondrial chloride channels, for example 108 pS. Finally, the high-conductance channel slightly selective for cations with an average conductance of 908 pS was observed. After addition of DIDS the channel activity was completely and irreversibly inhibited. Analysis of the electrophysiological specificity of this channel indicates its dissimilarity.

This work was supported by the Polish Mitochondrial Network.

doi:10.1016/j.bbabio.2010.04.399

16P.5 The kinetic and molecular mechanisms of the Zn^{2+}-activated mitochondrial swelling

Evgeniya V. Kravenska, Nataliya V. Fedirko
Ivan Franko National University of Lviv, Department of Human and Animal Physiology, Lviv, Ukraine
E-mail: k.evgeniya.v@gmail.com

It is known that Zn^{2+} activates the mitochondrial (Mit) permeability transition that accompanied with opening the permeability transition pore and the Mit swelling. The latter was frequently leading to the cell damage and death. Nevertheless the characteristics of the Zn^{2+}-induced Mit swelling remain elusive. The aim of this research was to study the kinetic and molecular mechanisms of the Mit swelling stimulated by Zn^{2+}. The experiments were performed on the isolated Mit obtained from the rat liver by differential centrifugation. Mit swelling was measured spectrophotometrically and analyzed by: (i) maximal rate; (ii) lag-time; (iii) peak amplitude. We showed, that Zn^{2+} (0.1–7.0 μM) activated Mit swelling. The increase of extra-Mit concentration of Zn^{2+} caused decrease in the lag-time, but did not induce significant changes in the amplitude of the Zn^{2+}-induced Mit swelling. The dependence of the rate of Mit swelling on extra-Mit concentration of Zn^{2+} was bell-shaped (parabolic) with the peak magnitude equal to 3.0 μM of extra-Mit Zn^{2+}. V_{max} and $K_{0.5}$ of the ascending branch of the parabola were 0.283 $A_{540}/min \times mg$ of protein and 0.219 μM accordingly. We also found, that cyclosporin A (10 μM, 1 min) completely inhibited the Zn^{2+} (0.1, 3.0 μM)-induced Mit swelling. In the presence of dithiothreitol (1 mM, 1 min) the maximal rate, lag-time and the peak amplitude of the Zn^{2+} (0.1, 3.0 μM)-induced Mit swelling were 65 ± 12 ($p \leq 0.01$) and $31 \pm 5\%$ ($p \leq 0.001$), 233 ± 54 ($p \leq 0.01$) and $371 \pm 95\%$ ($p \leq 0.05$), and 94 ± 8 ($p > 0.05$) and $93 \pm 8\%$ ($p > 0.05$) correspondingly. In the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (1 mM, 1 min) the maximal rate, lag-time and the peak amplitude of the Zn^{2+} (0.1, 3.0 μM)-induced Mit swelling were 126 ± 16 ($p \leq 0.01$) and $72 \pm 13\%$ ($p \leq 0.05$), 34 ± 8 ($p \leq 0.05$) and $39 \pm 11\%$ ($p \leq 0.05$), and 132 ± 10 ($p \leq 0.01$) and $108 \pm 8\%$ ($p \leq 0.05$), correspondingly. In the presence of CGP-37157 (1 μM, 1 min) the maximal rate, lag-time and the peak amplitude of the Zn^{2+} (0.1, 3.0 μM)-induced Mit swelling were 121 ± 5 ($p > 0.05$) and $74 \pm 6\%$ ($p \leq 0.01$), 84 ± 21 ($p > 0.05$) and $76 \pm 15\%$ ($p \leq 0.05$), and 84 ± 12 ($p \leq 0.05$) and $87 \pm 7\%$ ($p \leq 0.01$), correspondingly. Thus, our results demonstrated that Zn^{2+} stimulates Mit swelling. This process is mediated by opening the permeability transition pore and regulated by the Mit SH-, COOH-groups and the Na^+/Ca^{2+}-exchanger.

doi:10.1016/j.bbabio.2010.04.400