Mitochondria isolated from embryos of the crustacean *Artemia franciscana* lack the Ca^{2+}-induced permeability transition pore. Although the composition of the pore described in mammalian mitochondria is unknown, the impacts of several effectors on pore opening are firmly established. Notably, ADP, ATP and bongkrekic acid delay, while carboxyatractyloside hasten Ca^{2+}-induced pore opening. Here we report that adenine nucleotides decreased, while carboxyatractyloside increased Ca^{2+} uptake capacity in mitochondria isolated from *Artemia* embryos. Bongkrekic acid had no effect on either Ca^{2+} uptake or ADP–ATP exchange rate. Transmission electron microscopy imaging of Ca^{2+}-loaded *Artemia* mitochondria showed needle-like formation of electron-dense material in the absence of adenine nucleotides, and dot-like formation in the presence of ADP. Energy-filtered transmission electron microscopy identified the material to be rich in Ca^{2+} and phosphorus. Sequencing of the *Artemia* ANT protein revealed that it lacked the last ~100 amino acids from the carboxy-terminus, compared to the closest ANT homologue expressed in *Xenopus laevis*, the latter exhibiting a 78–80% homology to human ANT-1, -2, and -3 and bovine isoforms. Isolated liver mitochondria from *Xenopi* exhibited Ca^{2+}-induced permeability transition pore, sensitive to inhibition by cyclosporin A and bongkrekic acid. We propose that the atypical effects of ANT ligands on Ca^{2+} uptake capacity in mitochondria from *Artemia* embryos is a consequence of their truncated ANT isoform. This is also associated with insensitivity to bongkrekic acid and a unique pattern of Ca^{2+} precipitation in their mitochondrial matrix. We further postulate that this truncated ANT results in the absence of a Ca^{2+}-induced pore.

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18P.6 MAC induces mitochondrial fragmentation

Pablo M. Peixoto, Kathleen W. Kinnally
New York University College of Dentistry, Department of Basic Sciences, USA
E-mail: pmp6@nyu.edu

Accumulating literature associates mitochondrial dynamics with apoptosis, since regulation of either process has reciprocal effects. These processes seem to converge in formation of the mitochondrial apoptosis induced channel, MAC, which releases cytochrome *c* and triggers the degradation phase of apoptosis. While Bax and Bak, core components of MAC, were shown to interact with fusion and fission proteins, some studies also suggest proteins from the intermembrane space could leak to the cytosol and further promote mitochondrial fission during apoptosis. The temporal relationship between apoptosis induction, MAC formation and mitochondrial fragmentation was investigated by time lapse microscopy. MAC function was induced through staurosporine treatment and microinjection of tBid or cytochrome *c*. MAC formation and mitochondrial dynamics were monitored in Hela cells (clone 10) that stably express low levels of GFP-Bax and were transiently transfected with a pDsRed-2 plasmid. GFP-Bax relocation to mitochondria occurs during apoptosis and signals MAC formation, while pDsRed-2 expression shows mitochondrial structure as red fluorescence. Treatment with staurosporine and microinjection with tBid induced relocation of Bax and collapse of the mitochondrial network. The temporal relationship between these two events was further analyzed. Interestingly, pretreatment with iMAC2, a specific MAC blocker, protected against cell death and prevented mitochondrial fragmentation after tBid injection. Our results suggest a link exists between MAC formation and collapse of the mitochondrial network during apoptosis.

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18P.7 Reversible enhancement of succinate dehydrogenase subunit A, succinate receptor and uncoupling proteins' mRNA levels in the course of physiological stress related to the dynamics of succinate dehydrogenase activity

Marie A. Simonova[1], Marie N. Tutukina[2], Nataliya V. Khunderyakova[3], Anna A. Vasilieva[3], Eugen I. Maevsky[3], Marie N. Kondrashova[3]
[1]*Pushchino State University, Faculty for Biophysics and Biomedicine, Russia*
[2]*Institute of Cell Biophysics RAS, Department of Functional Genomics and Cellular Stress, Russia*
[3]*Institute of Theoretical and Experimental Biophysics RAS, Department of Energetics of Biological Systems, Russia*
E-mail: tomorrow_comes@bk.ru

The novel cytobiochemical method preserving *ex vivo* the native structure of mitochondrial network allows to reveal on a large scale the dynamics of succinate dehydrogenase (SDH) activity *ex vivo* corresponding to the magnitude of *in vivo* changes [1, 2]. Intensive adrenergic activation of the organism within the range of physiological immobilization stress (IS) created by putting rat into a box includes initial strong SDH activation after 30 min treatment and subsequent decrease in excessive activity displaying the active adaptation to excitation at 120 min. In order to penetrate the mechanism of SDH dynamics we have investigated the expression of catalytic SDH subunit (*sdha*) and major succinate receptor (*gpr91*) on mRNA levels. Also we have monitored the dynamics in expression of uncoupling proteins *ucp2* and *ucp3* under IS conditions. Total RNA fractions from rat liver and spleen were isolated by standard hot acid phenol extraction. cDNAs were generated using gene-specific primers and M-MuLV reverse transcriptase, with RNA probes as a negative control. Expression levels were monitored by qRT-PCR. For reference house-keeping genes of β-actin and 16S ribosomal RNA were used. Our results indicate that on molecular level SDH activation under IS occur even more strikingly. We have observed that during initial phase of physiological stress the mRNA levels of enzyme subunit (*sdha*) and succinate receptor (*gpr91*) were enhanced 7- and 3-fold respectively, and as animal became adopted to stress conditions, expression decreased practically to the control levels. Furthermore, 30 min immobilization led to the dramatic increase in *ucp2*-mRNA levels, up to 1000-fold in spleen and 5-fold in liver, followed by expression reduction after 120 min (on average 4-fold over control level in spleen and 2-fold in liver). The same dynamics was observed for UCP-3 expression in spleen. Uncoupling proteins can prevent excessive potential accumulation on the mitochondrial membrane under stress thus eliminating harmful consequences.

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18P.8 Visualization of mitochondrial nucleoids in HepG2 and INS-1E cells

Jan Tauber, Andrea Dlasková, Jitka Šantorová, Katarína Smolková, Petr Ježek
Institute of Physiology, Dept. 75, Academy of Sciences, Prague, Czech Republic
E-mail: tauber@biomed.cas.cz

Mitochondria possess their own DNA (mtDNA) — in humans a 16.6 kb circular double-stranded molecule, which encodes 13 essential subunits of respiratory chain complexes and ATP synthase. mtDNA is not “naked”

but is organized into specific nucleo-protein complexes called mitochondrial nucleoids. In order to visualize mt-nucleoids in HepG2 and INS-1E cells, we have prepared several constructs coding for conjugates of a mitochondrial transcription factor A (TFAM) or a Twinkle helicase and various fluorescent proteins and photoconvertible fluorescent proteins, including GFP, DENDRA2, PS-CFP2 and Eos. Constructs for Viral Power lentiviral expression system (Invitrogen) were prepared as well as the corresponding lentiviral particles, that were subsequently used for cell transductions. In addition, immunocytology of mt-nucleoids was performed with the aid of anti-TFAM antibodies. Using confocal microscopy we have found co-localizations of each TFAM-conjugated fluorophores with TMRE or mitochondria-addressed DSRed fluorescent protein contouring mitochondrial reticulum network. Furthermore, an influence of oxidative stress, various metabolic states (glycolytic and obligatory oxidative phosphorylation) and several stress factors were tested in order to access changes in mt-nucleoids organization.

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18P.9 The large dynamin-like GTPase Mgm1 in mitochondrial fusion

Michael Zick[1], Anja Schäfer[1,2], Stéphane Duvezin-Caubet[1,3], Walter Neupert[1], Andreas S. Reichert[1,2]
[1]*Adolf-Butenandt-Institut für Physiologische Chemie, Ludwig-Maximilians-Universität München, Germany*
[2]*CEF Makromolekulare Komplexe, Mitochondriale Biologie, Fachbereich Medizin, Goethe-Universität Frankfurt am Main, Germany*
[3]*Institut de Biochimie et Génétique Cellulaires, CNRS-Université Bordeaux2, France*
E-mail: michael.zick@med.uniuenchen.de

The mitochondrial network is constantly remodeled by fusion and fission events. To understand the molecular mechanisms underlying mitochondrial fusion is highly relevant in regard to its role in cell physiology and disease pathogenesis. It is particularly interesting, as the process of mitochondrial fusion is remarkable in several aspects compared to other characterized cellular membrane fusion events: (i) There are in total four membranes that need to be fused in a coordinated manner; and (ii) fusion of mitochondria is not mediated by SNARE proteins, but instead by large dynamin-like GTPases. In yeast, Mgm1 is the representative of this class in the inner mitochondrial membrane. It exits as two isoforms, one short (s-Mgm1) and one long (l-Mgm1). s-Mgm1 is created by a specific cleavage through the rhomboid protease Pcp1. Our results suggest that this specificity is not due to the cleaved hydrophobic region itself, but rather determined through an exosite. We hypothesize that the exceptional biogenesis pathway of Mgm1 plays a crucial role in the regulated proteolysis, involving a potential interplay between Pcp1 and the TIM23 translocase. As a balanced formation of both isoforms is crucial for mitochondrial fusion, we have analyzed the differential characteristics of the two isoforms. Both interact in homotypic as well as heterotypic manner, but are differentially distributed across the submitochondrial compartments. l-Mgm1 is preferentially found in cristae, while s-Mgm1 mainly resides in the inner boundary membrane. Surprisingly, it became apparent that a functional GTPase domain is only required in the short but not in the long isoform of Mgm1. These new findings let us propose that the two isoforms carry out distinct, presumably interrelated functions in mitochondrial fusion and cristae maintenance.

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