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

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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

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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

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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”