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matter of dispute. Recently, we identified a novel protein complex composed of a pore-forming core (MitoK) and of a regulatory subunit (mitoSUR) located in the inner mitochondrial membrane. In planar lipid bilayer experiments in 100 mM potassium gluconate solution, performed employing the recombinant mitoK protein alone, the conductance and kinetic behaviour of the observed activity resembled those of the previously described mitoKATP channel. However, activity was not inhibited by Mg^{2+} and ATP, unless MitoK and mitoSUR were co-expressed. These two proteins together formed a channel that was sensitive to mM concentration of ATP and was activated by diazoxide, a well-known pharmacological agonist of mitoKATP. Given the observed effect of several classical pharmacological activators and inhibitors of mitoKATP, our data suggest that these two proteins might be responsible, at least under certain conditions, for mitoKATP activity and for the control of mitochondrial ion homeostasis.

doi:10.1016/j.bbabio.2016.04.157

06.07

Modulation of the mitochondrial carnitine/acylcarnitine transporter by acetylation

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The mitochondrial carnitine/acylcarnitine transporter (CACT) catalyses carnitine/acylcarnitine antiport. Its function has been defined mainly in proteoliposome experimental models. Despite CACT represents a putative site of β-oxidation regulation, few data are available about its modulation. Lysine acetylation is a post-translational modification (PTM) of a huge number of proteins. It has been shown that iper-acetylation of long chain acyl CoA dehydrogenase (LCAD) impairs its enzymatic activity. It could be hypothesized that other components of the same pathway, such as CACT, could be regulated by a similar mechanism. Indeed, CACT is partially acetylated in rat liver as revealed by WB analysis using an anti-acetyl-Lys antibody. Acetylation can be reversed by the mitochondrial deacetylase SIRT3. After treatment of the mitochondrial extract with SIRT3, the CACT activity, assayed in proteoliposomes, increases with respect to the untreated control. The half-saturation constant is not influenced, while the Vmax is increased. The kinetic data suggests that steric hindrance of acetyl groups impairs conformational changes, rather than substrate binding. Recently, it was shown that acetylation of mitochondrial proteins also occur by a non-enzymatic pathway under conditions of reduced Acetyl-CoA buffering [1]. Recombinant CACT which is not acetylated was incubated with acetyl-CoA and then subjected to WB with anti-acetyl-Lys antibody and transport assay. Data showed that non-enzymatic acetylation of CACT occurs and impairs its activity. In conclusion, CACT is regulated by acetylation representing a control site of beta-oxidation pathway together with LCAD.

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doi:10.1016/j.bbabio.2016.04.158

06.08

Do dimers of ATP synthase form the PTP in pea stem mitochondria?

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In animal cells Ca^{2+} and ROS induce a sudden change in the inner mitochondrial membrane permeability, which has been named Permeability Transition (PT). Recently, it has been proposed that dimers of F-ATP synthase form the Permeability Transition Pore (PTP), the mega-channel involved in this phenomenon [1]. This feature has not yet been characterized in plants, even if their mitochondria possess the candidate components for PTP formation. Therefore, we characterized the functional properties of PTP in plant mitochondria and verified if F-ATP synthase possesses channel function in electrophysiology experiments. Mitochondria isolated from pea stem underwent PT when Ca^{2+} was added in the presence of the ionophore ETH129. The membrane electrical potential was then collapsed and the phenomenon matched by Ca^{2+} release but not by mitochondrial swelling. As is observed with the PT of animal mitochondria, Cyclosporin A (CsA) significantly delayed occurrence of PT, which was inhibited by Mg^{2+}-nucleotides and favored by benzodiazepine Bz-423 and oxidants, such as phenylarsine oxide and diamide. In electrophysiology experiments, F-ATP synthase dimers inserted into an artificial bilayer showed channel activity characterized by a rather small conductance, which could explain the inability of plant PTP to mediate mitochondrial swelling. These data suggest that F-ATP synthase is involved in PTP formation also in plant mitochondria.

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doi:10.1016/j.bbabio.2016.04.159

06.09

Modulation of mitochondrial adenine nucleotide translocase (ANT) regulation with ageing

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