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, performing 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 Mg2+ 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.


06.07

Modulation of the mitochondrial carnitine/acylcarnitine transporter by acetylation

Lara Consolea, Nicola Giangregorib, Annamaria Tonazzib, Cesare Indiverib

a Dipartmento DiBEST (Biologia, Ecologia, Scienze della Terra)Unit of Biochemistry and Molecular Biotechnology, University of Calabria, Via Bucci 4C, 87036 Arcavacata di Rende, Italy
b Dipartmento DiBEST (Biologia, Ecologia, Scienze della Terra)Unit of Biochemistry and Molecular Biotechnology, University of Calabria, Via Bucci 4C, 87036 Arcavacata di Rende, Italy

E-mail address: console@hotmail.it (L. Console)

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-acyetyl-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 LCAD. Non-enzymatic acetylation of CACT occurs and impairs its activity. In conclusion, CACT is regulated by acetylation representing a control site of β-oxidation pathway together with LCAD.

References


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06.08

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

Valentina De Cola, Carlo Peressonb, Elisa Petrussea, Valentino Casoloc, Sonia Patuia, Alberto Bertolini2, Valentina Giorgio3, Vanessa Checchetto1, Enrico Braidot1, Giovanna Lippe, Ildikó Szabó4, Angelo Vianello5, Paolo Bernardi6, Marco Zancani3

1 Dept. of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Italy
2 Consiglio Nazionale delle Ricerche Institute of Neuroscience and Department of Biomedical Sciences, University of Padova, Italy
3 Department of Biology, University of Padova, Italy
E-mail address: decol.valentina@spes.uniiud.it (V. De Col)

In animal cells Ca2+ 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 megachannel 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 Ca2+ was added in the presence of the ionophore ETH129. The membrane electrical potential was then collapsed and the phenomenon matched by Ca2+ 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 Mg2+-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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06.09

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

Philippe Dioletzc, Isabelle Bourdel-Marchassonn, Philippe Pasdoisa, Dominique Detaillle, Audrey Sémont, Richard Roulanda, Guillaume Calmetted, Gilles Gouspilloue

a INSERM U1045 - Centre de Recherche Cardio-Thoracique de Bordeaux & LIRYC - Institut de Rythmologie et Modélisation Cardiaque, Université de Bordeaux, CHU de Bordeaux, France
b CHU de Bordeaux, Pôle de gériatrie clinique, Bordeaux, France
c Résidence Magnétique des Systèmes Biologiques, UMR 5536 CNRS – Université de Bordeaux, France
d Département de Medicine (Cardiology), David Geffen School of Medicine, University of California, Los Angeles, CA, USA
e Département de Kinanthropologie, Université du Québec à Montréal, Montréal, QC, Canada
E-mail address: philippe.diolez@ihu-liryc.fr (P. Diolez)

In animal cells Ca2+ 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 megachannel 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 Ca2+ was added in the presence of the ionophore ETH129. The membrane electrical potential was then collapsed and the phenomenon matched by Ca2+ 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 Mg2+-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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Philippe Dioletz, Isabelle Bourdel-Marchasson, Philippe Pasdois, Dominique Detaillle, Audrey Sémont, Richard Rouland, Guillaume Calmette, Gilles Gouspillou

INSERM U1045 - Centre de Recherche Cardio-Thoracique de Bordeaux & LIRYC - Institut de Rythmologie et Modélisation Cardiaque, Université de Bordeaux, CHU de Bordeaux, France

CHU de Bordeaux, Pôle de gériatrie clinique, Bordeaux, France

Résidence Magnétique des Systèmes Biologiques, UMR 5536 CNRS – Université de Bordeaux, France

Département de Medicine (Cardiology), David Geffen School of Medicine, University of California, Los Angeles, CA, USA

Département de Kinanthropologie, Université du Québec à Montréal, Montréal, QC, Canada

E-mail address: philippe.diolez@ihu-liryc.fr (P. Diolez)