



HAL
open science

Tumor suppressor fumarate hydratase is phosphorylated and regulated by AMP-activated protein kinase

Anna Klaus, Cécile Polge, Sarah Zorman, Yolanda Auchli, René Brunisholz,
Uwe Schlattner

► To cite this version:

Anna Klaus, Cécile Polge, Sarah Zorman, Yolanda Auchli, René Brunisholz, et al.. Tumor suppressor fumarate hydratase is phosphorylated and regulated by AMP-activated protein kinase. 17. European Bioenergetics Conference, Sep 2012, Freiburg, Germany. , Biochimica et Biophysica Acta - Bioenergetics, 1817, pp.S85-S86, 2012, 2012 EBEC abstracts. 10.1016/j.bbabbio.2012.06.233 . hal-02747890

HAL Id: hal-02747890

<https://hal.inrae.fr/hal-02747890>

Submitted on 3 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

direct H₂O₂ activation of mt-iPLA2 γ and provide an important insight on the potential *in vivo* regulations of iPLA2 catalytic activities.

Reference

- [1] J. Ježek, M. Jabůrek, J. Zelenka, P. Ježek, *Physiol. Res.* 59 (2010) 737–747.

doi:10.1016/j.bbabbio.2012.06.230

11P5

A novel mitochondrial potassium channel in embryonic hippocampal mitochondria

A. Kajma, P. Bednarczyk, A. Szewczyk
*Nencki Institute of Experimental Biology,
 Laboratory of Intracellular Ion Channels, Pasteura 3,
 PL-02-093 Warsaw, Poland
 University Warsaw of Life Sciences,
 Department of Biophysics, Nowoursynowska 159,
 PL-02-776 Warszawa, Poland
 E-mail: a.kajma@nencki.gov.pl*

Since 1991, when in *Nature* Inoue and co-workers described the first potassium channel in the inner mitochondrial membrane, ATP-regulated (mitoK_{ATP}), four other channels were found. ATP-regulated (mitoK_{ATP}), large-conductance calcium activated (mitoBK_{Ca}), voltage-dependent (mitoKv1.3), and *intermediate-conductance calcium-activated* potassium channel (mitoIK_{Ca}), were identified by the electrophysiological techniques. The last, TWIK-related acid-sensitive potassium channel (TASK-3) was identified with the use of immunofluorescence methods.

Patch-clamp single channel studies on mitochondria isolated from embryonic rat hippocampus revealed the presence of the potassium channel which has outwardly rectifying activity at the symmetrical conditions (150 mM/150 mM KCl). The channel displayed a conductance of 61 pS, at positive voltages, and also strong voltage dependence. Patch-clamp studies at the mitoplast-attached mode showed that this channel was not sensitive to the classical activators and inhibitors of the mitochondrial potassium channels, but regulated by the pH and arachidonic acid.

In summary, by the single channel recordings, we characterized for the first time an ion channel which was cation selective, permeable to potassium ions and displayed voltage sensitivity. The channel does not correspond to the potassium ion channels described earlier in the inner mitochondrial membrane.

Grant sponsor: DEC-2011/01/N/NZ1/04311.

doi:10.1016/j.bbabbio.2012.06.231

11P6

Manganese superoxide dismutase controls T cell activation-induced oxidative signaling and cell death

Marcin M. Kamiński^{1,*,#}, Daniel Röth¹, Sabine Sass¹, Sven W. Sauer², Peter H. Krammer¹, Karsten Gülow^{1,*,#}

¹*Division of Immunogenetics (D030), Tumor Immunology Program, German Cancer Research Center (DFKZ), Im Neuenheimer Feld 280, Heidelberg, Germany*

²*Department of General Pediatrics, Division of Inborn Metabolic Diseases, University Children's Hospital, Heidelberg, Germany*

E-mail: m.kaminski@dkfz.de; k.guelow@dkfz.de

*These authors contributed equally to the work.

T cell receptor (TCR)-mediated generation of mitochondrial reactive oxygen species (ROS) plays a crucial role for expression of interleukin 2 (IL-2) and CD95 ligand (CD95L, FasL/Apo-1L) genes, and consequently, for T cell activation and activation-induced cell death (AICD). Our study demonstrates that a major mitochondrial antioxidative enzyme, manganese superoxide dismutase (MnSOD/SOD2), acts as an important control switch in the process of T activation-induced oxidative signal generation. Higher abundance and activity of MnSOD in the late phase of TCR-triggered response temporally associates with a shut-down phase of mitochondrial oxidative signal generation. Transient or inducible MnSOD over-expression inhibited mitochondrial ROS production. In turn, lower oxidative signal resulted in an abrogated NF- κ B- and AP-1-mediated transcription of IL-2 and CD95L genes and decreased IL-2 secretion as well as CD95L-dependent AICD. Moreover, TCR-mediated transcriptional upregulation of MnSOD demonstrates a negative feedback regulation being itself dependent on oxidation-sensitive transcription. Our finding stresses a critical role for MnSOD and mitochondria as regulators of T cell activation.

References

- [1] M.M. Kamiński, D. Röth, S. Sass, S.W. Sauer, P.H. Krammer, K. Gülow, *Biochim. Biophys. Acta* 1823 (5) (May 2012) 1041–1052.
 [2] M.M. Kamiński, S.W. Sauer, C.D. Klemke, D. Süß, J.G. Okun, P.H. Krammer, K. Gülow, *J. Immunol.* 184 (9) (May 1 2010) 4827–4841.
 [3] M. Kamiński, M. Kiessling, D. Süß, P.H. Krammer, K. Gülow, *Mol. Cell. Biol.* 27 (10) (May 2007) 3625–3639.
 [4] K. Gülow, M. Kamiński, K. Darvas, D. Süß, M. Li-Weber, P.H. Krammer, *J. Immunol.* 174 (9) (May 1 2005) 5249–5260.

doi:10.1016/j.bbabbio.2012.06.232

11P7

Tumor suppressor fumarate hydratase is phosphorylated and regulated by AMP-activated protein kinase

A. Klaus^{1,2,#}, C. Polge^{1,2,#}, S. Zorman^{1,2}, Y. Auchli³, R. Brunisholz³, U. Schlattner^{1,2}

¹*University Joseph Fourier, Laboratory of Fundamental and Applied Bioenergetics (LBFA) and Environmental and Systems Biology (BEeSy), Grenoble, France*

²*Inserm, U1055, Grenoble, France*

³*Functional Genomics Center Zurich, ETH Zurich/University of Zurich, Switzerland*

E-mail: uwe.schlattner@ujf-grenoble.fr

#Contributed equally.

AMP-activated protein kinase (AMPK) is a central cellular signaling hub involved in energy homeostasis, growth and proliferation. The kinase is considered as a promising target for pharmacological intervention in several energy-related pathologies like diabetes type II and cancer. However, its signaling network is still incompletely understood. Here we have applied a novel, two-dimensional *in vitro* screen to search for novel AMPK substrates [1]. By combining biophysical interaction based on surface 7pasmon resonance with *in vitro* phosphorylation, this screen can identify AMPK substrates that are specific for a given AMPK isoform. Application of this screen to full-length AMPK α 2 β 2 γ 1 and soluble rat liver proteins identified the tumor suppressor fumarate hydratase (FH). FH was confirmed to interact with and to be preferentially phosphorylated by the AMPK α 2 isoform using yeast-two-hybrid [2] and *in vitro* phosphorylation assays. AMPK-mediated phosphorylation of FH significantly increased enzyme activity *in vitro* and *in vivo*, suggesting that it is a *bona fide* AMPK substrate. *In vivo*, AMPK α 2 may target the cytosolic/

nuclear pools of FH, whose tumor suppressor functions rely on DNA damage repair and stabilization of HIF-1 α -signaling that induces pseudohypoxia.

References

- [1] A. Klaus, C. Polge, S. Zorman, Y. Auchli, R. Brunisholz, U. Schlattner, J. Proteomics 75 (2012) 3304–3313.
- [2] A. Brückner, C. Polge, N. Lentze, D. Auerbach, U. Schlattner, Int. J. Mol. Sci. 10 (2009) 2763–2788.

doi:10.1016/j.bbabbio.2012.06.233

11P8

Mitochondrial potassium channels in *Dictyostelium discoideum*

M. Laskowski¹, A. Kicinska², W. Jarmuszkiwicz², A. Szewczyk¹

¹Nencki Institute of Experimental Biology,

Laboratory of Intracellular Ion Channels, Warsaw, Poland

²Laboratory of Bioenergetics, Faculty of Biology,

Adam Mickiewicz University, Poznan, Poland

E-mail: m.laskowski@nencki.gov.pl

Mitochondria are crucial not only in energy metabolism but also in regulation of cell senescence and apoptosis. The strict control of inner mitochondrial membrane permeability and selective ion transport is essential for mitochondrial functioning. Potassium ion homeostasis is an important process for mitochondrial optimal functioning. Potassium channels such as ATP-regulated, large conductance calcium activated and voltage dependent channels were observed in inner mitochondrial membrane in various mammalian tissues. Recently, we have identified potassium channels in inner mitochondrial membrane of potato *Solanum tuberosum* and *Acanthamoeba castellanii*. Currently we characterize mitochondrial potassium channels from one of *Dictyostelium* species. It is commonly used as a model organism to study cell differentiation, metabolism and programmed cell death. Preliminary experiments are focused on biophysical and pharmacological characterization of mitochondrial ion channels. Purified inner mitochondrial membranes (submitochondrial particles) were reconstituted into planar lipid bilayer. To form model membranes asolectin from soybean mixture of phospholipids was used. We observed two types of potassium selective ion channels in submitochondrial particle samples: a large- and small-conductance channels. Experiments were performed both in gradient solution 50/150 mM KCl (cis-trans) and in symmetrical solution 150/150 mM KCl at voltages from –50 to 50 mV. Regulation of the channel activity by divalent cations such as Ca²⁺ and Mg²⁺ was explored. Additionally, interaction of the ATP with mitochondrial potassium channels was characterized. The knowledge on mitochondrial ion channels may contribute to understanding molecular mechanism of *Dictyostelium discoideum* functioning.

This work was supported by Polish Mitochondrial Network MitoNet.pl.

doi:10.1016/j.bbabbio.2012.06.234

11P9

Reactive oxygen species in proinflammatory response of endothelial cells

A. Lukasiak¹, A. Zgrzywa², A. Wrzosek², A. Szewczyk²

¹Department of Biophysics, Warsaw University of Life Sciences SGGW, 159 Nowoursynowska St., 02-776 Warsaw, Poland

²Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, 3 Pasteura St., 02-093 Warsaw, Poland
E-mail: agnieszka_lojek@sggw.pl

Endothelium is a thin layer of cells lining all cardiovascular system. It plays crucial role in such diseases as atherosclerosis, hypertension and diabetes. It is well documented that the inflammation is responsible for these diseases, however very little is known about the role of mitochondria in development of proinflammatory state. It is known that reactive oxygen species (ROS) produced by mitochondria can be involved in the proinflammatory process.

As a model of our study we used endothelial immortalized cell line EA.hy 926 and as a marker molecule the ICAM-1 (intracellular adhesion molecule 1) expression was measured using flow cytometry method. The inflammation was induced by cytokine TNF- α (tumor necrosis factor α). We examined the role of reactive oxygen species (ROS) in the proinflammatory process using fluorescent probe DCF-DA. The main source of ROS production in cells is mitochondria, therefore we checked the effect of rotenone, the complex I of respiratory chain inhibitor, on ROS level in EA.hy 926 cells.

In our study TNF- α caused time and dose dependent increase of ICAM-1 from hardly detected residual level. Additionally our results show that TNF- α increases ROS production in EA.hy 926 cells in dose dependent manner. Rotenone was ineffective in changing the ROS production level in EA.hy 926 cells. Our results related to rotenone are slightly different to literature data that suggests that different cellular models can response to rotenone in different ways.

This work was supported by the European Union from the resources of the European Regional Development Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

doi:10.1016/j.bbabbio.2012.06.235

11P10

NCLX, but not Letm1, mediates matrix Ca²⁺ extrusion and modulates the mitochondrial redox state during HeLa cell stimulation

Umberto De Marchi^{1,2}, Jaime Santo-Domingo², Israel Sekler³, Andreas Wiederkehr¹, Nicolas Demaurex²

¹Nestlé Institute of Health Sciences, EPFL Campus, Bâtiment G, Quartier de l'Innovation, CH-1015 Lausanne, Switzerland

²Department of Cell Physiology and Metabolism, University of Geneva, rue Michel-Servet, 1, CH-1211 Genève, Switzerland

³Department of Physiology, Ben-Gurion University of Negev, Beer-Sheva, 84105, Israel

E-mail: umberto.demarchi@rd.nestle.com

Mitochondria sense and shape intracellular Ca²⁺ signals, acting as a cell signaling hub. The uptake of Ca²⁺ into the mitochondrial matrix activates intermediary and energy metabolism, and Ca²⁺ extrusion mechanisms ensure that this Ca²⁺ signal is transient. After a long quest, the proteins promoting Ca²⁺ uptake and release have been discovered: a mitochondrial Ca²⁺ uniporter was shown to mediate Ca²⁺ uptake, and two ion exchangers, NCLX and Letm1, were proposed to exchange Ca²⁺ against Na⁺ or H⁺ respectively.

To relate mitochondrial Ca²⁺ extrusion to mitochondrial function, we have manipulated the expression levels of NCLX and Letm1 and measured by single cell imaging their impact on matrix Ca²⁺, matrix redox state, and NAD(P)H concentration evoked by Ca²⁺ mobilizing agonists.

We find that the histamine stimulated mitochondrial Ca²⁺ rise is highly variable in individual HeLa cells. The rate of Ca²⁺ extrusion is a function of this amplitude, being highest for large matrix Ca²⁺