18L.4 Multi-site control and regulation of mitochondrial energy production

G. Benard, N. Bellance, C. Jose, S. Melser,
K. Nouette-Gaulain, R. Rossignol
INSERM U688, Physiopathologie mitochondriale,
Université Victor Segalen-Bordeaux 2, 146 rue Léo-Saignat,
F-33076 Bordeaux Cedex, France
E-mail: rossig@u-bordeaux2.fr

With the extraordinary progress of mitochondrial science and cell biology, novel biochemical pathways have emerged as strategic points of bioenergetic regulation and control. They include mitochondrial fusion, fission and organellar motility along microtubules and microfilaments (mitochondrial dynamics), mitochondrial turnover (biogenesis and degradation), and mitochondrial phospholipids synthesis. Yet, much is still unknown about the mutual interaction between mitochondrial energy state, biogenesis, dynamics and degradation. Meanwhile, clinical research into metabolic abnormalities in tumors as diverse as renal carcinoma, glioblastomas, paragangliomas or skin leiomyomata, has designated new genes, oncogenes and oncometabolites involved in the regulation of cellular and mitochondrial energy production. Furthermore, the examination of rare neurological diseases such as Charcot–Marie–Tooth type 2a, Autosomal Dominant Optic Atrophy, Lethal Defect of Mitochondrial and Peroxisomal Fission, or Spastic Paraplegia suggested involvement of MFN2, OPA1/3, DRP1 or Paraplegin, in the auxiliary control of mitochondrial energy production. Lastly, advances in the understanding of mitochondrial apoptosis have suggested a supplementary role for Bcl2 or Bax in the regulation of mitochondrial respiration and dynamics, which has fostered the investigation of alternative mechanisms of energy regulation. Here, we discuss the regulatory mechanisms of cellular and mitochondrial energy production, and we emphasize the importance of the study of rare neurological diseases in addition to more common disorders such as cancer, for the fundamental understanding of cellular and mitochondrial energy production.

doi:10.1016/j.bbabio.2010.04.420

18L.5 Role of the mitochondrial kinase Pink1 in Parkin recruitment and mitophagy

Richard J. Youle
National Institutes of Health, Bethesda, MD, USA
E-mail: youler@ninds.nih.gov

Pink1 is mutated in some autosomal recessive forms of Parkinson's disease and has been linked genetically to Parkin activity and to mitochondrial dysfunction. Recently we found that Parkin, a cytosolic E3 ligase, translocates to mitochondria upon mitochondrial damage or uncoupling. How Parkin may sense mitochondrial uncoupling, however, was unknown. Pink1, a kinase located on the outer mitochondrial membrane is strictly required for Parkin translocation to mitochondria and for Parkin-mediated mitophagy. The mechanism of Pink1 activation upon mitochondrial damage occurs by an unexpected posttranslational induction process. We identified a constitutive proteolytic processing and rapid turnover of endogenous Pink1 leading to low basal expression on healthy mitochondria. Upon mitochondrial uncoupling or damage, Pink1 proteolysis is prevented leading to a dramatic upregulation of Pink1 expression on mitochondria. Furthermore, Pink1 upregulation occurs selectively on individual impaired mitochondria within a milieu of energetically functional mitochondria in individual cells. We thus identify a novel mechanism for detection of mitochondrial function in cells and propose that lesions in a mitochondrial quality control pathway may contribute to some forms of parkinsonism.

doi:10.1016/j.bbabio.2010.04.421

Posters

18P.1 Mitochondria, autophagy and nitric oxide are correlated and control myogenesis

Clara De Palma[1], Serena Pisoni[1], Sarah Pambianco[1], Sestina Falcone[4], Luca Scorrano[2], Silvia Brunelli[3], Emilio Clementi[1]
[1]*Unit of Clinical Pharmacology "Luigi Sacco" Hospital of Milano, Italy*
[2]*Department of Cell Physiology and Metabolism,*
University of Geneva, Switzerland
[3]*Department of Experimental Medicine, University of Milano Bicocca, Italy*
[4]*Inserm, Institute de Myologie Univ Paris 06, France*
E-mail: clara.depalma@guest.unimi.it

Mitochondrial morphology is regulated by the balanced processes of fission and fusion; whether these events play a role also in shaping cell functions is still not well understood. Here we provide evidence that during myogenic differentiation the short mitochondria of myoblasts change into the extensively elongated network observed in myotubes. We show that mitochondrial elongation is required for myogenesis to occur and that this event depends on the cellular generation of nitric oxide (NO). Inhibition of NO synthesis in myogenic precursor cells leads to inhibition of mitochondrial elongation and of myogenic differentiation. The specific target of NO action is Drp1. NO controls Drp1 translocation to mitochondria and its docking to the specific interactor Fis1; further, it controls the GTPase activity of the protein. In the presence of NO Drp1 functions are maintained at basal levels to allow mitochondria to create a fused network that enhances myogenic differentiation. The action of NO on Drp1 is mediated through a direct phosphorylation of the protein, induced by activation of PKG. Inhibition of the NO pathway leads to mitochondrial fission accounting for a latent mitochondrial dysfunction, reducing respiratory activity and OXPHOS ATP production. Despite the apoptotic features of these mitochondria, myoblasts do not undergo spontaneous apoptosis. They acquire sensitivity to apoptosis in the presence of 3-methyl-adenine, a general inhibitor of lysosomes degradation. In addition we found a decrease of mitochondrial number and DNA when NO generation was inhibited, accompanied by increases in LC3 positive autophagosomes and cleavage of LC3. These findings suggest that mitochondrial latent dysfunction is related to induction of a mitophagic prosurvival pathway. In this study we demonstrate a new regulation of Drp1 function mediated by NO and the fundamental role played by this regulation during myogenic differentiation. In addition we show how mitophagy can act as a protective factor in the absence of NO. These results unravel a fine control check of the myogenic differentiation programme dependent on metabolic signals.

doi:10.1016/j.bbabio.2010.04.422

18P.2 Imaging of mitochondrial network and mt nucleoids in β-cells of diabetic Goto Kakizaki rats

Andrea Dlasková[1], Tomáš Špaček[1], Lydie Plecitá-Hlavatá[1],
Jitka Šantorová[1], BerkovaZuzana Berková[2], Jan Saudek[2], Petr Ježek[1]
[1]*Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i, Czech Republic*
[2]*Institute of Clinical and Experimental Medicine, Czech Republic*
E-mail: dlaskova@biomed.cas.cz

The morphology of mitochondrial network is highly variable and there is a substantial body of evidence for close relationship between cellular physiology and mitochondrial network organization. We set out to investigate whether pathology of diabetic β-cells is reflected by the altered morphology of mitochondrial network. Conventional confocal microscopy does not provide sufficient *z* axial resolution to realistically visualize 3D mitochondrial network, and therefore we applied high resolution 4Pi microscopy with *z* axial resolution of about 100 nm. Matrix-addressed GFP was lentivirally expressed in Langerhans islets isolated from diabetic Goto Kakizaki or control wistar rats. We demonstrate that β-cells within the Langerhans islets from diabetic Goto Kakizaki rats exhibited more disintegrated mitochondrial network compared to those from control Wistar rats. Observed average diameter of mitochondrial tubule was 236 ± 27 nm in Goto Kakizaki and 214 ± 16 nm in control rats. Our next aim was to characterize organization of mt nucleoids in primary β-cells of Langerhans Islets. To visualize mt nucleoids we used immunocytochemistry or lentiviral expression of GFP-labeled TFAM protein, which is known to have a crucial role in assembling of mammalian mt DNA.

doi:10.1016/j.bbabio.2010.04.423

18P.3 Mitochondrial fusion is an early and protective step of autophagy

Lígia C. Gomes[1,2], Luca Scorrano[1,3]
[1]*Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy*
[2]*PhD Program in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal*
[3]*Department of Cell Physiology and Metabolism, University of Geneva Medical School, Geneva, Switzerland*
E-mail: ligiag@cnc.cj.uc.pt

Autophagy is a catabolic process that allows the recycling of components of the cell under for instances conditions of nutrient depletion. Autophagy has been long regarded as an unselective process, but under some circumstances specific organelles like mitochondria are selectively engulfed by autophagosomes. We therefore explored whether mitochondrial morphological changes were associated with the onset of autophagy. Induction of autophagy led to mitochondrial elongation both *in vitro* and *in vivo*. Mitochondrial elongation correlated with increased fusion rate and required the core mitochondrial fusion proteins, as substantiated by a genetic analysis. A combination of real time imaging and the use of a pharmacological inhibitor indicated that cAMP-PKA axis mediates starvation-triggered mitochondrial elongation by blocking translocation of the pro-fission protein DRP1 to mitochondria. Elongation protected against mitophagy and was essential in the maintenance of ATP levels during periods of starvation. Ablation of the required pro-fusion genes converted mitochondria into sinks for ATP and caused starvation-induced death, showing a protective role for these morphological changes during periods of limited substrate supply. Thus, mitochondrial shape changes play an important role in the regulation of the fate of cells undergoing autophagy.

doi:10.1016/j.bbabio.2010.04.424

18P.4 Changes in sympathetic and parasympathetic regulation connected with succinate dehydrogenase and α-ketoglutarate dehydrogenase activity in different physiological states of the organism

Nataliya V. Khunderyakova, Marina V. Zakharchenko, Andrey V. Zakharchenko, Marie A. Simonova, Anna A. Vasilieva, Olga I. Romanova, Nadezda I. Fedotcheva, Elena G. Litvinova, Aleksandr A. Azarashvili, Eugen I. Maevsky, Marie N. Kondrashova
Institute of Theoretical and Experimental Biophysics RAS, Department of Energetics of Biological Systems, Russia
E-mail: butyanova@rambler.ru

The study was carried out by a novel cytobiochemical method highly sensitive to changes *in vivo* due to preservation *ex vivo* the native structure of mitochondrial network [1]. Succinate dehydrogenase (SDH) and α-ketoglutarate dehydrogenase (KDH) activity was measured in glass-adhered lymphocytes in smear of blood by nitro-blue-tetrazolium reduction [1]. The set of selected maximally identical, premature (6 week) male rats (6–8) was investigated simultaneously. The following states were investigated: intact state, after adrenaline (ADR) administration, under emotional immobilization stress in a box, newborn, premature and soon after maturation, depending on mother suckling, and under the action of biologically active substances. Healthy volunteers and patients with hypertension were also examined. The quiescent state in all cases is characterized by low activities of SDH and KDH well balanced with a very small prevalence of KDH over SDH. Together with increase in influence of exogenous or endogenous ADR activation of SDH per 200–400% and more is observed. This is accompanied initially by activation of KDG too, but to a lesser extent, while under more strong ADR action KDH activity falls dramatically. The state of highly active SDH without controlling action of KGL oxidation is non-stable and transforms into inhibition of both dehydrogenases under progression of influence. The moderate SDH activation is accompanied by increase in succinate-dependent Ca^{2+} accumulation in mitochondria, while hyperactivation is connected with its fall. Phases of activation and hyperactivation are respectively connected with elevation and lowering of immune function of neutrophils. Under strengthening of cholinergic regulation, related to maturation of animal, in contrast, increase in KDG activity and diminishing of SDH activation by ADR were observed. The data obtained demonstrate on a larger scale the discovery in isolated mitochondria of unification of sympathetic and parasympathetic regulation in the single system with oxidation of only the two substrates — succinate and α-ketoglutarate and explain the finding receptors also to only these two intermediates of oxidation [1].

This work was supported by the Federal program “Scientific and scientific pedagogical staff of the innovation Russia” (project no. 02.740.11.0312).

Reference

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doi:10.1016/j.bbabio.2010.04.425

18P.5 Mitochondria from *Artemia franciscana* embryos exhibit a truncated form of ant, associated with atypical effects of its ligands on Ca^{2+} uptake capacity and unique morphology of matrix Ca^{2+} precipitates

Csaba Konràd[1], Gergely Kiss[1], Beata Töröcsik[1], János L Lábár[2], Akos A. Gerencser[3], Miklós Mándi[1], Vera Adam-Vizi[1], Christos Chinopoulos[1]
[1]*Department of Medical Biochemistry, Semmelweis University, Budapest, 1094, Hungary*
[2]*Research Institute for Technical Physics and Materials Science, Budapest, 1121, Hungary*
[3]*Buck Institute for Age Research, Novato, CA, 94945, USA*
E-mail: csaba.konrad@gmail.com