Biochimica et Biophysica Acta 1797 (2010) 55–65

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Abstracts

S5 Mitochondria, Reactive Oxygen Species and Ageing

Lectures

5L.1 Substrate-dependence of mitochondrial reactive oxygen species generation

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Production of reactive oxygen species (ROS) is inherent to mitochondrial oxidative metabolism and numerous sources of ROS have been revealed in mitochondria. ROS production capacity is controlled by factors affecting and reflecting the metabolic state of intact mitochondria; among them the chemical nature of the substrates fuelling the respiratory chain and the amplitude of the membrane potential of mitochondria. In the presence of FAD-reducing substrates electrons reduce coenzyme Q and when the mitochondrial membrane potential is high, electrons can flow back to complex I (reverse electron transport; RET) and reduce NAD^+ to NADH. RET is associated with high rate of ROS generation. In mitochondria supported by NAD^+-linked substrates, NADH/NAD^+ ratio is critical for ROS generation by both complex I and α-ketoglutarate dehydrogenase. A common conception is that calcium overload leads to stimulated ROS generation in mitochondria. However, data in the literature are controversial; some supporting, others arguing against this. For the effect of calcium on ROS generation in isolated mitochondria the choice of substrate and the metabolic state of mitochondria are critical. In succinate-supported well-coupled mitochondria ROS emission is decreased by calcium due to the depolarization-related elimination of RET. With NAD^+-linked substrates, in the absence of induction of permeability transition pore (PTP), highly polarized mitochondria exhibiting high rate of ROS generation respond to a calcium load with a decreased ROS generation, whereas in depolarized mitochondria actively synthesizing ATP, the effect of calcium depends on the amount of calcium load and could result in either no change or stimulation of ROS generation reflecting the membrane-potential-dependent character of ROS formation. In mitochondria favoring calcium-induced PTP, ROS emission from mitochondria is dominated by PTP-related permeability increase of the inner membrane.

doi:10.1016/j.bbabio.2010.04.179

5L.2 Control of ROS production and T-cell turnover by the p13 protein of HTLV-1

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The present study was aimed at gaining insight into the function of p13, an 87-amino acid mitochondrial protein expressed by HTLV-1. Although necessary for viral propagation in vivo, the mechanism of p13 function is incompletely understood. In previous studies we showed that p13 exerts antitumor effects in experimental transformation models. More recently, using synthetic p13 and isolated mitochondria, we showed that the protein triggers an inward K^+ current that leads to mitochondrial depolarization, increased activity of the respiratory chain, and reactive oxygen species (ROS) production. These findings prompted us to test the effects of p13 on ROS in living cells, including T-cells, the main targets of HTLV-1 infection in vivo. Expression of p13 in primary T-cells resulted in cell activation, measured using the CD38 surface marker. p13-induced activation was blocked in the presence of ROS scavengers and was not observed using a p13 mutant that was inactive in the in vitro assays, indicating a connection between the effects on ROS those on mitochondrial K^+ influx. In the context of the transformed cell line Jurkat, p13 did not affect ROS levels unless the cells were subjected to glucose deprivation, which led to a p13-dependent increase in ROS and cell death. Using RNA interference we confirmed that expression of p13 also influences glucose starvation-induced cell death in HTLV-1-infected cells. Taken together, our findings indicate that in the context of the HTLV-1 propagation strategy, p13 could increase the pool of "normal" infected cells while culling cells acquiring a transformed phenotype, thus favoring life-long persistence of the virus in the host.

doi:10.1016/j.bbabio.2010.04.180

5L.3 Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species

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This review highlights the important role of redox signaling between mitochondria and NADPH oxidases. Besides the definition and general importance of redox signaling, the cross-talk between mitochondrial and Nox-derived reactive oxygen species (ROS) is discussed on the basis of 4 different examples. In the first model, angiotensin-II is discussed as a trigger for NADPH oxidase activation with subsequent ROS-dependent opening of mitochondrial ATP-

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sensitive potassium channels leading to depolarization of mitochondrial membrane potential followed by mitochondrial ROS formation and respiratory dysfunction. This concept was supported by observations that ethidium bromide-induced mitochondrial damage suppressed angiotensin-II-dependent increase in Nox1 and oxidative stress. In another example hypoxia was used as a stimulator of mitochondrial ROS formation and by using pharmacological and genetic inhibitors, a role of mitochondrial ROS for the induction of NADPH oxidase *via* PKCε was demonstrated. The third model was based on cell death by serum withdrawal that promotes the production of ROS in human 293T cells by stimulating both the mitochondria and Nox1. By superior molecular biological methods the authors showed that mitochondria were responsible for the fast onset of ROS formation followed by a slower but long-lasting oxidative stress condition based on the activation of an NADPH oxidase (Nox1) in response to the fast mitochondrial ROS formation. Finally, a cross-talk between mitochondria and NADPH oxidases (Nox2) was shown in nitroglycerin-induced tolerance involving the mitochondrial permeability transition pore and ATP-sensitive potassium channels. The use of these redox signaling pathways as pharmacological targets is briefly discussed.

doi:10.1016/j.bbabio.2010.04.181

5L.4 The significance of thermogenesis for the ageing process

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Impaired thermogenesis is one of the features of ageing. Activity and recruitment of mitochondria in brown adipose tissue is important for the thermogenic needs of animals. Therefore thermogenesis, both at the level of brown-fat mitochondria and at the intact animal level, was examined here in PolgA mtDNA polymerase mutant mice (mtDNA mutator), a mouse strain exhibiting numerous mutations of mtDNA and several features of premature aging. At the mitochondrial level, as compared with wild-type mitochondria, on all three substrates investigated (pyruvate, palmitoyl-l-carnitine and glycerol-3-phosphate), UCP1-dependent oxygen consumption was significantly reduced in mutant mitochondria, similarly to maximal oxidative capacity (FCCP-response), indicating impaired thermogenesis. In intact mice, at environmental temperatures below 20 °C, mtDNA mutator mice were unable to further increase their metabolism and went into torpor. Response to adrenergic stimulus (NE injection) was significantly reduced in mtDNA mutator mice. Thus, mtDNA mutation led to lower activity of brown-fat mitochondria and impaired thermogenesis; i.e. also in this respect, mtDNA. Mutator mice mimicked normal ageing. Maintaining the mice at thermoneutral temperature (30 °C) ameliorated many of the ageing symptoms. Remarkably, the life span of the mice at thermoneutrality was increased by around 100 days, emphasizing the significance of thermogenesis for the ageing process.

doi:10.1016/j.bbabio.2010.04.182

5L.5 Mitochondrial fatty acid oxidation and oxidative stress: Lack of reverse electron transfer-associated production of reactive oxygen species

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Lipotoxicity results from accumulation of lipids in non-adipose tissue and is partly attributed to an impairment of mitochondrial physiology by elevated level of nonesterified fatty acids (FFA). Typical activities of FFA are uncoupling and modulation of cellular ROS generation. Here, we examined the likely ability of fatty acid oxidation to initiate ROS generation by reverse electron transfer (RET). RET from succinate to NAD^+ is known to be accompanied by high generation of reactive oxygen species (ROS). In contrast, oxidation of fatty acids by mitochondria, despite being a powerful source of $FADH_2$, does not exhibit RET-associated ROS generation. Oxidation of carnitine esters of medium- and long-chain fatty acids by rat heart mitochondria is accompanied by neither high level of $NADH/NAD^+$ nor intramitochondrial reduction of acetoacetate to β-hydroxybutyrate, comparable to those accompanying succinate oxidation, although it produces the same polarization of the inner mitochondrial membrane, evidenced by high transmembrane potential ($\Delta\psi$). Also in contrast to the oxidation of succinate, where conversion of the pH difference between the mitochondrial matrix and the medium into $\Delta\psi$ by addition of nigericin results in a decrease of ROS generation, the same treatment during oxidation of octanoylcarnitine produces a large increase of ROS. Analysis of respiratory chain complexes by Blue Native polyacrylamide gel electrophoresis revealed bands that could tentatively point to supercomplex formation between complexes II and I and complexes II and III. However, no such association could be found between complex I and the electron transferring flavoprotein that participates in fatty acid oxidation. It is speculated that structural association between respective respiratory chain components may facilitate effective RET.

doi:10.1016/j.bbabio.2010.04.183

5L.6 Mitochondrial Ca^{2+} and ROS crosstalk signaling

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Mitochondria are central to cellular energy metabolism as the source of much of the cell's ATP, as well as being a hub for cellular Ca^{2+} and redox signaling pathways. Mitochondrial Ca^{2+} and morphology are effectors of ATP synthesis, yet Ca^{2+} overload and morphological deformation can lead to mitochondrial dysfunction and ultimately cell death. Moreover, Ca^{2+} uptake by mitochondria is actively involved in shaping cellular Ca^{2+} dynamics by regulating the concentrations of Ca^{2+} within microdomains between mitochondria and Ca^{2+} transporters existing in nearby sarco/endoplasmic reticulum and plasma membranes. Reactive oxygen species (ROS) are generated, at least in part, as a consequence of ATP production in the mitochondria and are important for cellular signaling, yet contribute to oxidative stress and cellular damage. ROS are important for regulating the activity of redox sensitive enzymes and ion channels within the cell, including Ca^{2+} channels. For both Ca^{2+} and ROS, a delicate balance exists between the beneficial and detrimental effects on mitochondria. In this presentation, I bring together current data on mechanisms of mitochondrial Ca^{2+}-mediated ROS generation and mitochondrial fission. I propose a model for crosstalk between Ca^{2+} and ROS signaling pathways within mitochondrial microdomains, focusing on the role of mitochondrial fission machinery and mitochondrial permeability transition pores as a modulator of Ca^{2+} dynamics and ROS generation.