Biochimica et Biophysica Acta 1797 (2010) 119–131

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Abstracts

S15 Cell Physiology, Apoptosis and Mitochondrial Signalling

Lectures

15L.1 Dissecting the BAK-driven outer mitochondrial membrane permeabilization pathway using *in vitro* reconstituted systems

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BAK is a key proapoptotic member of the BCL-2 family, which primarily functions by forming an apoptotic pore in the mitochondrial outer membrane (MOM) allowing for the release of multiple deadly factors into the cytosol. Under healthy conditions, BAK adopts an inactive, "non-permeabilizing" conformation at the MOM. Upon apoptosis triggering, BAK interacts with specific components of the MOM and shifts to an activated "permeabilizing" state. However, the precise molecular pathway by which BAK proapoptotic function is activated remains ill defined, and the biophysical mechanism of pore formation by BAK remains unclear. We examined these issues using a combination of biophysical and biochemical techniques in an *in vitro* reconstituted system consisting of recombinant purified proteins and MOM-like liposomes. Using this minimalistic system we demonstrate that BAK possesses an intrinsic pore-forming activity, which can be unleashed by sequential interaction with selected mitochondrial lipids and proteins. The BAK-driven liposome permeabilization pathway is associated with membrane insertion and oligomerization of the protein, and culminates with formation of a proteolipidic pore which gradually grows in size. We also present information on the topography adopted by BAK at distinct stages along the membrane permeabilization pathway.

doi:10.1016/j.bbabio.2010.04.355

15L.2 Mitochondria in regulation of cell death in cardiovascular and brain pathologies

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Mitochondrial membrane permeabilization has been considered as the central, irreversible event in mitochondrial pathway of apoptosis. Opening of mitochondrial permeability transition pore (MPTP) is one of the means of permeabilization of mitochondrial membranes leading to the release of cytochrome *c* from mitochondria, caspase activation and apoptotic or necrotic cell death. MPTP has been implicated in various pathologies including heart ischemia/reperfusion, beta amyloid-induced neurotoxicity in Alzheimer's disease, etc. In such pathologies, inhibition of MPTP is critical for cardio- and neuroprotection. We have shown that low concentrations of NO may activate signalling pathways involving activation of protein kinase C and protein kinase G (PKG) and leading to increased resistance of mitochondria to opening of MPTP. Similarly, high physiological concentrations of estrogens trigger signalling cascades involving Akt, NO synthase, PKG and rendering mitochondria increased resistance to calcium-induced MPTP and protecting against ischemia-induced apoptosis in the heart. In the contrary to previous beliefs, there is accumulating evidence that mitochondrial cytochrome *c* release does not necessarily lead to cell death. If cells have high ability to reduce cytosolic cytochrome *c*, then caspase activation can be blocked even at post-cytochrome *c* level. We and others have shown that reduced cytochrome *c* is less capable in caspase activation than its oxidized form. Now we demonstrate that cytochrome *c* reducing agents such as TMPD can prevent ischemia-reperfusion-induced caspase activation and apoptosis in perfused heart or in neuronal cell cultures exposed to staurosporin or oligomeric forms of beta amyloid. Mitochondria also posses activities which can oxidize or reduce cytosolic cytochrome *c* and their external cytochrome *c*-reducing activity may depend on cytosolic concentrations of NAD(P)H.

doi:10.1016/j.bbabio.2010.04.356

15L.3 pH Spikes in individual mitochondria

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Mitochondria are multifunctional intracellular organelles involved in energy production, apoptosis, and calcium signalling. The pH within the mitochondrial matrix (pH_{mito}) is an important bioenergetic parameter that has been well studied in isolated mitochondria. How pH_{mito} is regulated in intact living cells is less clear and all *in situ* studies so far report spatially averaged measurements of pH_{mito}. In this study, we use a new pH-sensitive GFP-based fluorescent probe targeted to the mitochondrial matrix, mito-SypHer, to study pH_{mito} homeostasis at the level of single mitochondria. In Hela cells expressing mito-SypHer, we observed that individual mitochondria undergo spontaneous basifications transients, pH spikes. The pH spikes occurred randomly in time and space and had a characteristic profile, with a rapid onset (time to peak 1.6 ± 0.1 sec), a slower decay (t=8.5 ± 0.6 sec), and an average amplitude of 0.38 ± 0.05 pH units. Spikes were not spatially restricted to single mitochondria but were also observed in clusters of interconnected mitochondria, suggesting

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