Biochimica et Biophysica Acta 1837 (2014) e51–e56

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

S5 – Biogenesis of respiratory complexes

S5.L1

Regulation of protein homeostasis in the intermembrane space of mitochondria

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The vast majority of mitochondrial proteins are synthesized in the cytosol. After synthesis, precursors of mitochondrial proteins are directed to the TOM complex and subsequently to specific mitochondrial import machineries that are responsible for their sorting. Proteins destined to the intermembrane space of mitochondria follow the mitochondrial intermembrane space import and assembly (MIA) pathway. The MIA pathway provides an efficient mechanism for trapping substrate proteins in the intermembrane space of mitochondria. Our recent findings on dynamic processes that contribute to the maintaining of protein homeostasis in this mitochondrial compartment will be discussed.

doi:10.1016/j.bbabio.2014.05.141

S5.L2

The role of mitochondrial biogenesis and ROS in the control of energy supply in proliferating cells

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In living cells, growth is the result of coupling between substrate catabolism and multiple metabolic processes that can be assumed to take place during net biomass formation and maintenance processes. We have shown that in yeast, there is a constant growth yield during proliferation on non-fermentable substrate (where the ATP generated originates from oxidative phosphorylation). This constant growth yield is due to a tight adjustment between the growth rate and the cellular mitochondrial amount. Two processes are known to control the cellular steady state of mitochondrial amount: mitochondrial biogenesis and mitochondrial degradation. Whereas mitochondrial degradation (mitophagy) is unaffected during proliferation, mitochondrial amount is strictly controlled by mitochondrial biogenesis. Moreover, the Ras/cAMP pathway is the key cellular signaling pathway involved in the regulation of mitochondrial biogenesis, with a direct relationship between the activity of this pathway and the cellular amount of mitochondria. The yeast harbors three cAMP kinase catalytic subunits, which have greater than 75% identity and are encoded by the TPK genes. Although they are redundant for some functions it is not the case for all of them. We have shown that Tpk3p is the catalytic subunit specifically involved in the regulation of mitochondrial biogenesis. Indeed, Δptk3 cells exhibit an oxidative stress that originates in an increase in mitochondrial ROS production due to the loss of cAMP dependent regulation of mitochondrial ROS production. This was the first report showing that the activity of the HAP complex is sensitive to ROS signaling, clearly involving ROS in mitochondria-to-nucleus signaling. This ROS-induced decrease in the amount of HAP complex is due to a ROS-induced decrease in the amount of Hap4p (functional homologue of PGC1α in mammalian cells). In conclusion, mitochondria being one of the main sites of ROS production, the ROS-mediated downregulation of the mitochondrial biogenesis can be considered like a protective quality-control process. We also showed that the glutathione redox state is an intermediary for the sensing of cell redox stress by the transcription factors involved in the mitochondrial biogenesis regulation. Post-translational consequences of cellular redox perturbation most likely consist in a main regulatory processes and the question of the relationship between the cell proliferation and its redox status is a highly discussed research field.

doi:10.1016/j.bbabio.2014.05.142

S5.L3

Coupling of mitochondrial biogenesis to oxidative phosphorylation through the ATP-dependent activity of Bcs1

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Complex III of the mitochondrial respiratory chain consists of 11 or 10 different subunits in mammals and yeast, respectively, of which three are catalytic: cytochromes b, c1 and the FeS protein Rip1. Complex III is assembled through a sequential addition of subunits, starting with an early core containing Cytb, the only subunit encoded by the mitochondrial genome and finishing with the incorporation of Rip1, a step that is dependent on the assembly factor Bcs1. Bcs1 proteins belong to the large AAA-protein family (ATPase Associated with diverse cellular Activities) characterized by the presence of a highly conserved AAA region involved in ATP binding and hydrolysis. Mutations in the AAA domain of Bcs1 prevent Bcs1 from efficiently hydrolyzing ATP and lead to the accumulation of an inactive pre-complex III lacking Rip1. Mutations in the human gene BCS1L are associated with very different clinical presentations ranging from mild to lethal pathologies. We have identified several classes of extragenic compensatory mutations able to

restore the assembly of complex III in yeast cells mutated in the AAA domain of Bcs1. Unexpectedly, the first class of compensatory mutations mainly target the mitochondrial ATP synthase, leading to a strong decrease in the ATP hydrolysis activity while maintaining a sufficient level of ATP synthesis to sustain respiratory growth. We propose that by reducing ATP hydrolysis by the ATP synthase, the compensatory mutations increase the concentration of ATP in mitochondria, thereby increasing the ATP hydrolysis activity of the mutated Bcs1p and allowing it to recover its chaperon function.

doi:10.1016/j.bbabio.2014.05.143

S5.L4

Molecular bases for the complex I stability dependency from other respiratory complexes

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In mammalian mitochondria, complex I becomes unstable in the absence of complexes III (1) or IV (2, 3). On the contrary, the absence of complex I does not affect substantially the stability of the other respiratory complexes. By systematic analysis of the degradation process of complex I in cellular models of CIII or CIV ablation, we have identified the signals that trigger it, and defined the molecular mechanism responsible for the stability of complex I. Finally we have investigated the potential physiological role of this interdependency.

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doi:10.1016/j.bbabio.2014.05.144

S5.O1

High molecular weight forms of mammalian respiratory chain complex II

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Supercomplexes of mammalian mitochondrial respiratory chain formed by complexes I, III, and IV are well established. In contrast, the involvement of succinate dehydrogenase, complex II (CII), linking respiratory chain with tricarboxylic acid (TCA) cycle, in supramolecular structures remains questionable. To search for higher molecular weight forms of CII and specific interactions of CII with other complexes of oxidative phosphorylation (OXPHOS) pathway or with TCA cycle we combined mild detergent solubilisation of mitochondria and different types of native electrophoresis. For experiments we used rat tissues and different cell lines of murine and human origin including fibroblasts with different OXPHOS defects or cells devoid of mtDNA. Mitochondrial proteins were solubilised with digitonin, separated by native electrophoresis or two-dimensional electrophoretic systems and detected by immunoblotting or in-gel assay of activities of OXPHOS complexes. For immunoprecipitation of CII and ATP synthase (complex V, CV) we used antibodies to SDHA subunit of CII and antibodies to F1 subunits of CV.

We have found that digitonin-solubilised complex II quantitatively forms high molecular weight structures (CII_{hmw}) that can be resolved by clear native electrophoresis. CII_{hmw} structures are enzymatically active and differ in electrophoretic mobility between tissues (500–over 1000 kDa) and cultured cells (400–670 kDa). Whilst their formation is unaffected by isolated defects in other respiratory chain complexes, they are destabilised in mtDNA-depleted rho0 cells. Molecular interactions responsible for the assembly of CII_{hmw} are rather weak with the complexes being more stable in tissues than in cultured cells. Whilst our electrophoretic studies and immunoprecipitation experiments of CII_{hmw} do not indicate specific interactions with the respiratory chain complex I, III or IV or enzymes of the tricarboxylic acid cycle, they point out to a specific interaction between CII and ATP synthase [1].

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doi:10.1016/j.bbabio.2014.05.145

S5.O2

Impaired mitochondrial energetic function and altered supramolecular interactions of respiratory chain complexes in cells bearing a novel pathogenic cytochrome b microdeletion

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Cytochrome b is the only mtDNA-encoded subunit of the respiratory complex III (ubiquinol: cytochrome c oxidoreductase; CIII), the central component of the respiratory chain. In its native form, CIII is dimeric and is closely associated in varying proportions with CI and CIV to form supramolecular structures, referred to as supercomplexes or "respirasomes". The occurrence of such supercomplexes as structural and functional entities is well documented. This indicates that interactions among CI/CIII and CIII/CIV are dynamic events, allowing cells to adapt their respiratory activity to specific cell-type requirements. This concept is also important in relation to human diseases caused by mitochondrial dysfunctions. Defects in CIII are relatively rare and mostly associated with mutations in the MTCYB gene. It has been shown that mutations in this gene can result in CIII deficiency alone or can produce combined CI and CIII failure, likely as a consequence of the critical role of CIII in the stability of CI in respirasomes. Here, we report a novel heteroplasmic mtDNA micro-deletion in MTCYB gene, identified in a patient suffering a