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The membrane potential is a key regulator for oxidation-dependent protein import into the mitochondrial inter membrane space (IMS)
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The respiratory chain of mitochondria produces the main amount of the cellular ATP. To maintain the function of this machinery most of its proteins have to be imported into mitochondria after their synthesis in the cytosol. Although most soluble mitochondrial proteins contain a mitochondrial targeting signal (MTS), many of the proteins of the IMS do not contain a MTS but instead are imported by an oxidative folding mechanism [1,2]. Examples of such proteins are the so called twin-CX_{9C} proteins that contain four conserved cysteines. After translocation into the IMS two intramolecular disulfide bonds are formed which contribute to the correct folding and the retention of the proteins [3]. The machinery that facilitates oxidation is composed of two main components, the essential disease-related proteins Mia40 and ALR. Mia40 is an oxidative reductase that interacts with the reduced and unfolded substrate proteins and oxidizes them. Mia40 is reduced after this reaction, and it is subsequently regenerated by ALR [4]. This pathway has so far been almost exclusively characterized in yeast cells and on isolated mitochondria. We aim to characterize the pathway and its regulation in intact mammalian cells. In our experiments we could verify the importance of Mia40 and ALR for import and assembly of proteins of the twin-CX_{9C}-family in intact cells. Surprisingly the import machinery of Mia40 and ALR is quite robust and unaffected by several mitochondrial stress conditions like the inhibition of respiratory chain complexes and limited oxygen availability. However, contrary to in vitro data, the oxidative protein import depends on the membrane potential; exhibiting similarities to the MTS-dependent protein import. Despite the dependence on the proton gradient, both import pathways exhibit significant differences in the kinetics of import. Import and processing of MTS-containing proteins is a fast process that occurs co-translationally or in a very fast posttranslational manner. Conversely, the substrates of Mia40 remain soluble in the cytosol after synthesis, and are imported in a slow process that is limited by the rate of Mia40-mediated oxidation.

References

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Biogenesis of mitochondria connects to the cell cycle
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While the pathogenic mechanisms of Parkinson's disease (PD) remain elusive, increasing evidence suggests that neurodegeneration in PD is associated with impaired mitochondrial 'quality control' — an imbalance between biogenesis and removal by autophagy. PD has also been associated with impaired mitochondrial complex I activity and complex I inhibitors like the neurotoxin rotenone cause pathological and neurochemical changes with a remarkable similarity to PD [1]. Rosiglitazone is a member of thiazolidinediones (TZDs), used clinically as insulin-sensitizing drugs. These compounds selectively bind to peroxisome proliferator-activated receptor gamma (PPARg) a nuclear receptor and ligand-dependent transcription factor used clinically as insulin-sensitizing drugs. These compounds selectively bind to peroxisome proliferator-activated receptor gamma (PPARg) a nuclear receptor and ligand-dependent transcription factor and carbohydrate metabolism, upregulates antioxidant defences and promotes mitochondrial biogenesis and has anti-inflammatory properties [2]. PPARg agonists provide protection in several in vitro and in vivo models of neurodegenerative disorders [3,4], but the mechanism of protection is not clear. We have studied whether rosiglitazone prevents mitochondrial damage of differentiated human neuroblastoma SH-SY5Y cells exposed to rotenone and have explored the interaction of biogenesis, autophagy and oxidative stress pathways in neuroprotection. Our data show that chronic partial and complex I inhibitors like the neurotoxin rotenone cause increases mitochondrial biogenesis and decreases autophagy and free radical generation. All these changes were reversed by co-treatment of the cells with rosiglitazone, which increased mitochondrial biogenesis and decreased autophagy and free radical generation. Our data indicate that rosiglitazone is potentially neuroprotective, acting directly on mitochondrial function in neurons, and not indirectly by suppressing inflammation, and might provide a valuable therapeutic strategy for the treatment of progressive neurodegenerative disease such as Parkinson's disease.

References

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Mitochondria still possess some features telling of their endosymbiotic origin like their own DNA and fission/fusion machineries. As the cell grows and increases its size, the need for energy feeds back via nuclear transcription regulation in order to upregulate genes involved in mitochondrial biogenesis [1]. As more than 99% of all mitochondrial proteins are encoded in the nucleus, they are translated on cytosolic ribosomes and post-translationally imported into the organelle. This process starts at an essential protein complex in the outer membrane of mitochondria, called Translocase of the Outer Membrane or TOM complex [2].

In a recent study [3] we identified several phosphorylated residues in this complex and found that mitochondria are much more subjected to cellular signalling cascades than previously thought [4,5]. The mitotic state of the cell is reflected in the activity of cyclin-dependent kinases (CDK), and indeed, at least one TOM protein has been identified as a target for this group.

This study now focuses on the role of CDK-dependent phosphorylation of Tom. We demonstrate that the yeast CDK phosphorylates Tom6 specifically during the M Phase of the cell cycle. This phosphorylation influences the TOM complex composition under conditions, when mitochondrial biogenesis needs to be stimulated.

References

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Adaptation of respiratory chain biogenesis to cytochrome c oxidase deficiency caused by SURF1 gene mutations
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Mutations in SURF1 gene leading to loss of an assembly protein Surf1 are frequent cause of severe defects of cytochrome c oxidase (COX). Clinical manifestation of these mutations is Leigh syndrome — a fatal neurodegenerative disease. Surf1 appears to be involved in an early step of COX assembly but its exact function remains unknown. In our study we focused on alteration of COX assembly process as a consequence of SURF1 mutations, on differences in supercomplex (SC) formation due to decreased amount of COX, and how SURF1 mutations influence protein and transcript levels of oxidative-phosphorylation system (OXPHOS) and other pro-mitochondrial genes. For experiments we used fibroblast cell lines from 9 patients with different SURF1 gene mutations and control cells. Proteins solubilised from inner mitochondrial membrane with mild detergents were analyzed using blue native electrophoresis in combination with SDS PAGE and Western blot detection. Transcript levels were determined with Agilent 44 k human genome microarray technique.

Decreased COX level (to 30%) in patient’s mitochondria was accompanied with upregulation of complexes I, III and V (130–150%) and accumulation of Cox5a subunit. Whole genome expression profiling showed general decrease of transcriptional activity in patient’s cells and indicated that observed changes in OXPHOS complexes have to be due to posttranscriptional compensatory mechanisms.

Fully assembled and functional COX was present mainly in I–III2–IV SC in patient mitochondria, while in control mitochondria the content of COX in SCs was comparable to that of free COX monomer. Lack of COX in patients further led to accumulation of basic I–III2 SC form, complex III dimer and COX assembly intermediates. Using 2D electrophoresis we identified two comigrating COX assembly intermediates in the 85–130 kDa region. One was the originally proposed S2 intermediate consisting of Cox1, 4, and 5a subunits and the other one contained large amount of Cox1 subunits. It could represent Cox1 associated with other unknown proteins but almost no Cox4 and Cox5a subunits. Both intermediates were completely unable to associate with complexes I and III into SCs [1].

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Reference

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Two pentatricopeptide repeat proteins are essential for biogenesis of the NADH:ubiquinone oxidoreductase from the filamentous fungus Neurospora crassa
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The NADH:ubiquinone oxidoreductase is the first and largest complex of the mitochondrial respiratory chain. Its assembly involves subunits encoded by the mitochondrial as well as the nuclear genome and requires biogenesis factors, which are not part of the mature complex I. Pentatricopeptide repeat (PPR) proteins are known to be involved in various steps of gene expression in organelles [1]. Eight genes encoding PPR proteins are found in Neurospora crassa (N. crassa). We determined the relevance of these genes for the assembly of complex I by characterizing the corresponding knockout mutants obtained by a high-throughput programme [2]. The identification of complex I defects was accomplished by measuring the NADH:ferricyanide redox activity and Blue Native polyacrylamide gel electrophoresis. Two knockout mutants were specifically affected in the assembly of complex I. The presence of a peripheral arm and the absence of a detectable membrane arm were demonstrated. Assembly intermediates were detected by western blot analysis using specific polyclonal antibodies against different complex 1 subunits. Moreover, a specific influence of the PPR proteins on processing respectively on amounts of mitochondrial RNA was observed by means of Northern Blots. To characterize their specific impact on biogenesis of complex I, the two proteins were expressed heterologously in Escherichia coli (E. coli) and purified via immobilized metal ion affinity chromatography (IMAC) and size