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AUTHOR'S VIEW

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Author's view: a nuclear transcription factor relocalizing to mitochondria rescues cells from proteotoxic aggregates

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ABSTRACT

Mitochondrial proteostasis is essential for survival, and imbalances can result in severe human diseases. We identified a novel stress response triggered upon accumulation of proteotoxic aggregates in the mitochondrial matrix. Mitochondria-to-nucleus signaling results in a transcriptional response and translocation of a nuclear transcription factor into mitochondria to maintain mitochondrial gene expression.

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Mitochondria fulfill several crucial cellular functions from energy supply, synthesis of heme, amino acids or lipids to programmed cell death. As 99% of all mitochondrial proteins are encoded in the nucleus, changes in nuclear transcription shape the mitochondrial proteome and can adapt it to changing cellular demands. Similarly, upon imbalances in the mitochondrial proteome, mitochondria-to-nucleus signaling can elicit transcriptional changes to restore mitochondrial homeostasis. Several mitochondria-to-nucleus communication pathways have been described that are activated by various perturbances in mitochondrial proteostasis, bioenergetics or metabolism.[1,2] One of the first identified responses is provoked by accumulation of unfolded proteins that exceed the capacity of the chaperone network and was therefore termed mitochondrial unfolded protein response (mtUPR). Initially identified in mammalian cells, several studies on mtUPR have also been performed in *C. elegans*.[3–5] However, mechanistic insights into mtUPR activation, signaling and the chronological order of events are still missing.

We recently identified patients with mutations in PMPCB, the catalytic subunit of the essential mitochondrial processing protease (MPP), which result in severe neurodegeneration in early childhood.[6] MPP cleaves the targeting signals from newly imported precursor proteins after their translocation into the matrix. These N-terminal presequences are found in approximately 70% of all mitochondrial precursors. Unprocessed precursors were anticipated to be less stable and rapidly degraded resulting in destabilization of the mitochondrial proteome.[7] Introduction of PMPCB patient mutations into the homologous Mas1 protein in *Saccharomyces cerevisiae* resulted in a temperature sensitive phenotype, in which MPP activity was impaired upon growth at elevated temperature.[6,8] These cells enabled not only investigation of the cellular consequences of defective MPP processing but also analysis of the fate of unprocessed precursors. Upon MPP inactivation unprocessed precursors accumulated in the mitochondrial matrix. Surprisingly, instead of being degraded the precursors were aggregating directly after import behaving completely opposite as proposed. Our finding that mitochondria fail to efficiently degrade aggregated precursors also raises the question how these organelles can degrade cytosolic misfolded proteins specifically transported into mitochondria for turnover as reported previously.[2]

Besides the formation of aggregates, we made two further intriguing observations: MPP inactivation did not result in increased cell death, but in a strong increase in protein levels of the mitochondrial chaperone Hsp10. This suggested that mitochondria-to-nucleus signaling upon MPP dysfunction triggered a protective response to increase the mitochondrial protein folding capacity. Transcriptomic analysis revealed a strong transcriptional response already two hours after MPP inactivation. At this early time-point, only a very minor fraction of unprocessed proteins accumulated and the mitochondrial proteome represented by mature functional proteins was not yet affected. Nevertheless the cells executed a strong transcriptional response, increasing mitochondrial and also cytosolic chaperones (Figure 1). We wondered if the transcriptional response could be part of an mtUPR, whose existence in yeast was still under debate[1] and assessed further characteristics of mitochondrial stress responses. However, none of the classical stress parameters identified in previous studies was changed in our model: Protein import was unaltered, we did not measure a change in membrane potential ($\Delta\psi$), ATP levels or reactive oxygen species (ROS), and neither cytosolic nor mitochondrial translation was affected. We asked if the transcriptional response resulted in the maintenance of these crucial functions and aimed to

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identify its mediator. We systematically screened several candidates, but only deletion of the nuclear transcription factor Rox1 resulted in exacerbation of the MPP mutant growth defect. However, when assessing nuclear transcripts the response was not depending on Rox1. Nevertheless all mitochondrial parameters were deteriorating in the absence of Rox1: The membrane potential (Δψ) was decreasing, ATP levels declined and protein import was compromised, ROS were increasing and cytosolic translation decreasing. Moreover, MPP mutant cells lacking Rox1 underwent increased cell death.

How was the nuclear transcription factor Rox1 mediating the protective effects if not via changes in nuclear transcription? Surprisingly, survival of the MPP mutant cells was not only dependent on Rox1, but also on presence of mitochondrial DNA (mtDNA). We wondered if the protective effects of Rox1 and mtDNA were based on the same mechanism and assessed Rox1 localization upon proteotoxic stress. Indeed, Rox1 was transported into mitochondria upon MPP dysfunction (while nuclear Rox1 levels remained equal). Rox1 belongs to the family of HMG (high mobility group) box containing proteins with the most prominent member TFAM (mitochondrial transcription factor A). Human TFAM plays an important role in maintenance and expression of the mitochondrial genome.[9] We wondered if Rox1 could have a TFAM-like function. Rox1 could not only bind mtDNA, but was also crucial to stabilize newly synthesized mtDNA in the MPP mutant cells. Furthermore, Rox1 was strongly increasing mitochondrial transcription upon MPP dysfunction resulting in the maintenance of mitochondrial translation (Figure 1).

Taken together, identification of an mtUPR in yeast enabled us to dissect for the first time the early steps in proteotoxic stress responses. In our model, mitochondria-to-nucleus signaling results in a rapid transcriptional response upon formation of proteotoxic aggregates and also triggers the translocation of the nuclear transcription factor Rox1 to mitochondria. This is required to protect mtDNA and maintain mitochondrial genome expression, which is essential for cell survival. While most of the so far employed mtUPR triggers likely perturb mitochondrial protein import,[4] our early response strictly depends on maintenance of this mitochondrial function to execute the first protective line of defense. Previous studies reporting the translocation of transcription factors into the nucleus as consequence of impaired protein import (e.g. ATFS-1 or ATF5) upon mtUPR therefore likely represent later events.[4,5] Employing a temperature-sensitive mutant enabled us to induce mild and reversible stress on the protein folding network that allowed identification of the earlier response upon proteotoxic stress. The identification of this protective mechanism might also be valuable to understand onset and progression of human diseases that are associated with dysfunctions in mitochondrial proteome maintenance as all involved proteins are highly conserved.

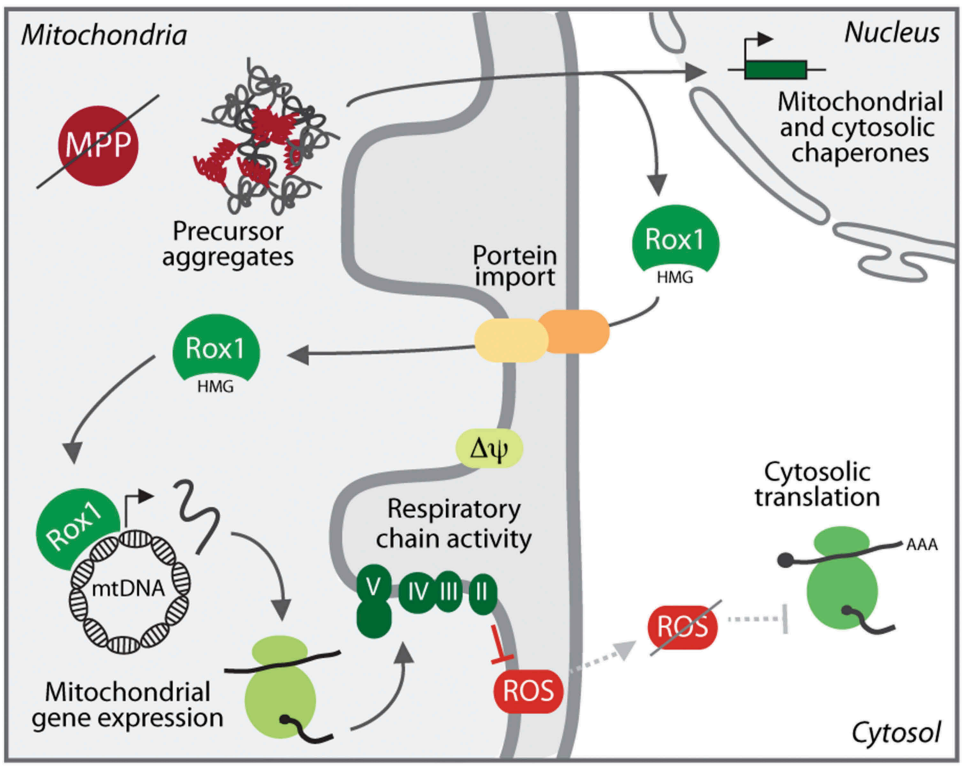

Figure 1. Relocalization of the nuclear transcription factor Rox1 protects cells from proteotoxic aggregates in the mitochondrial matrix. Dysfunction of the mitochondrial presequence protease MPP results in accumulation of unprocessed precursor proteins in the matrix that rapidly aggregate. This proteotoxic stress triggers mitochondria-to-nucleus signaling that results in a nuclear transcriptional response and also translocation of the nuclear transcription factor Rox1 into mitochondria. Rox1 binds to mitochondrial DNA (mtDNA) and maintains expression of the mitochondrial genome. This is pivotal for a functional respiratory chain, which generates a stable membrane potential (Δψ) critically to maintain protein import and decreases formation of reactive oxygen species (ROS), which would result in decreased cytosolic translation.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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