TITLE: MitoCPR: Meticulous Monitoring of Mitochondrial Proteostasis

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mitoCPR: meticulous monitoring of mitochondrial proteostasis

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Summary

Mitochondrial protein import stress compromises functioning of the organelles, due to inadequate supply of inner mitochondrial proteins. Weidberg and Amon describe a new monitoring pathway in budding yeast which restores mitochondrial function following the clearing of accumulated unfolded pre-transported mitochondrial proteins, by devising a molecular strategy of overexpressing bi-partite containing mitochondrial proteins.

Main Text

Balanced mitochondrial function is core to cellular health. Mitochondrial quality control encompasses processes ranging from timely protein degradation to full organelle removal via autophagy (mitophagy). Mitochondrial deficiencies drive signaling cues that communicate with the nucleus to mitigate lasting damage caused by various stressors. For instance, deficits in the import of proteins into mitochondria trigger the unfolded protein response (mtUPR) that, via retrograde signaling pathways, primes nuclear gene transcription. Protective and preventative retrograde signaling mechanisms comprising mPOS (mitochondrial precursor over-accumulation stress) and UPRam (unfolded protein response activated by mistargeting of proteins) are primarily cytoplasmic stress responses, i.e. not directly responsible for protecting mitochondrial function. In budding yeast Weinberg and Amon describe a new mechanism, mitoCPR (mitochondrial compromised import response) (Weidberg and Amon, 2018), which reinstates mitochondrial function post import stress by clearing the accumulated precursor proteins, and paving the way for adequate supply of newly transcribed proteins to be appropriately placed inside the mitochondria.

The authors demonstrate that, in partnership with MSP-1 (Mitochondrial Sorting of Proteins-1), the expression of CIS1 (human SOCS1; suppressor of cytokine signalling) induced by the transcription factor PDR3 (Pleiotropic Drug Resistance 3) clears and degrades ectopically accumulated or untransported mitochondrial pre-proteins. PDR3, interestingly, also mediates the multidrug resistance (MDR) response. Classically, the MDR is a highly conserved (from prokaryotes to mammals) molecular pathway that includes many proteins of unknown function as well as the ATP-Binding Cassette family of membrane proteins. These ABC proteins are pumps which facilitate the efflux of detrimental chemical insults (Liesa et al., 2012). The overexpression of mito-importable proteins, in particular bipartite-signal-containing proteins such as PSD1, induces a transcription profile that mimics that of the ancient MDR response. Hence, MitoCPR may represent an evolutionarily conserved mechanism that monitors the import machinery, preventing overwhelming stress and guarding mitochondria function through an efflux-based resistance mechanism.

The phenomenon of MitoCPR is observed both by using uncouplers of mitochondrial membrane potential ($\Delta\Psi_m$) (e.g. FCCP, CCCP) as well as by modulating the ‘protein

import stressor' directly. By driving uncontrolled overexpression of wild type PSD1 (*wt* phosphatidylserine decarboxylase-1), as well as PSD1 lacking the MTS (mitochondrial targeting sequence), it is revealed that MitoCPR is induced by ectopic accumulation of inner-membrane targeted bipartite element containing proteins, as measured by assessment of the level of COX5a^{pre}, the unfolded precursor of inner mitochondrial membrane (IMM) protein COX5a (Cytochrome-c oxidase 5 subunit A) on the Outer Mitochondrial Membrane (OMM). MitoCPR is also observed to be activated in cells with inherently compromised mitochondrial import such as *Rho0* cells, or *TAM41*$^{-/-}$ cells, confirming the importance of MitoCPR in combating mitochondrial import defects. Collectively, the observations introduce MitoCPR as a nuanced and specific monitoring modality, which now occupies a prominent space in the gamut of mitochondrial quality control mechanisms.

The human analogue of Msp1, ATAD1 (ATPase Family, AAA Domain Containing-1), has a mitochondrial/nuclear distribution in the cell and has previously been described to have a related function. ATAD1 is able to limit the accumulation of tail-anchored (TA) proteins on the mitochondria and when its function is compromised, there is overall mitochondrial dysfunction and loss of mtDNA (mitochondrial DNA) (Chen et al., 2014). However, not much is known about its interacting partners or whether ATAD1 can generate a MitoCPR response similar to Msp-1 in yeast. The findings by Weinberg and Amon suggest that in yeast MitoCPR can rescue mitochondrial function, begging for a more detailed exploration of ATAD1 and its partner proteins in mammalian cells. Furthermore, deficits in mitochondrial import have been implicated in human disease (Harbauer et al., 2014; Yano et al., 2014) and understanding rescue mechanisms can play a crucial role in elucidating pathology. Deficits in mitochondrial protein import directly impact the supply of newly transcribed proteins required for mitochondrial function. Exploring the structure and function of the MitoCPR in mammalian cells can open up the possibility of restoring mitochondrial function during pathological import-stress.

The strategy described by Weinberg and Amon, of measuring accumulation of precursor proteins on the OMM, can therefore serve as a read-out in mammalian cells to study the contribution of ATAD1 and related proteins towards hMitoCPR (human MitoCPR).

On a deeper mechanistic level, the regulation of the MitoCPR remains elusive. How does import stress signal to activate PDR3 and its downstream transcription of genes? Recent evidence suggests that mitochondria can regulate their own pruning via activation of lysosomal biogenesis, which in turn results in mitophagy (Fernandez-Mosquera et al., 2017). It may be interesting to then ask whether the inducers of mitochondrial biogenesis also induce MitoCPR as a parallel monitoring mechanism. Equally relevant would be to learn whether MitoCPR is induced exclusively after the accumulation of unimported elements on the OMM or also during the process of mitochondrial expansion following biogenesis enabled by mito-nuclear retrograde signaling.

In any case, this manuscript advances our understanding of the stress-induced mechanisms of mitochondrial proteostasis and retro-communication revealing that a crowded OMM signals to enact a clean-up mechanism to regain organelle function and hence cellular homeostasis.

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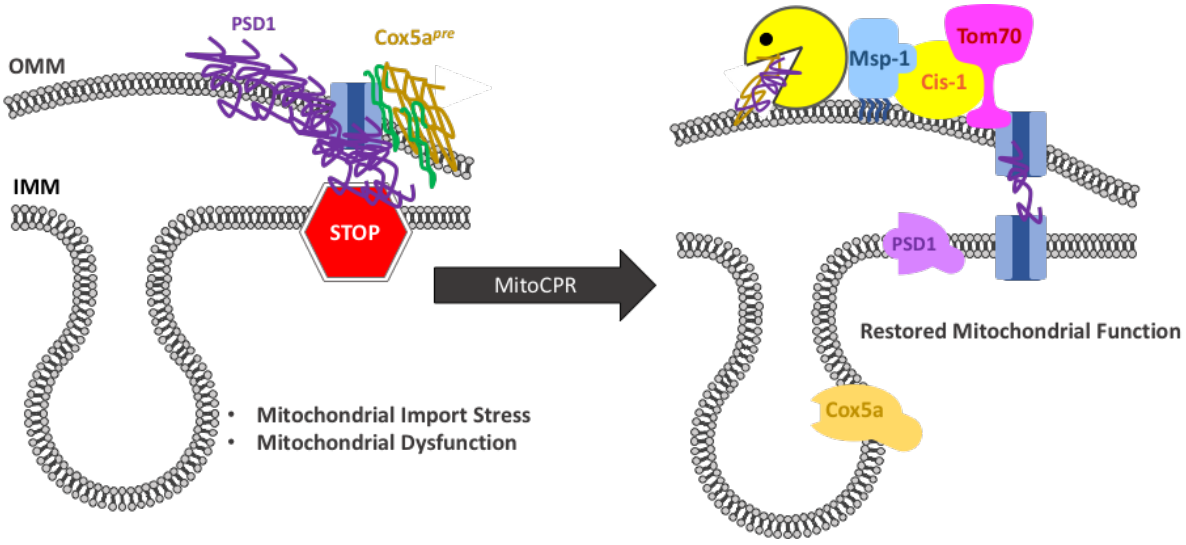
Legend to Figure 1: MitoCPR restores mitochondrial function after import stress by facilitating clean-up.

During mitochondrial import stress, as simulated via upregulation of bipartite signal containing protein PSD-1 (purple), the mitochondrial outer membrane (OMM) accumulates unfolded proteins. The resultant mitochondrial dysfunction is a reflection

of inadequate supply of imported proteins such as COX5a (ochre, used as a readout in *Weinberg and Amon (Science 2018*). Cis-1 (yellow) which interacts with Tom70 (pink) as well as Msp-1 (blue) assembles a protein complex on the OMM which triggers the proteosomal degradation (PAC-MAN®) of ectopically accumulated proteins from the OMM, restoring mitochondrial function.

Figure 1


PSD1
Cox5a^pre
OMM
IMM
STOP
MitoCPR
• Mitochondrial Import Stress
• Mitochondrial Dysfunction
Msp-1
Tom70
Cis-1
PSD1
Restored Mitochondrial Function
Cox5a