

## ATF4 helps mitochondria pass the stress test

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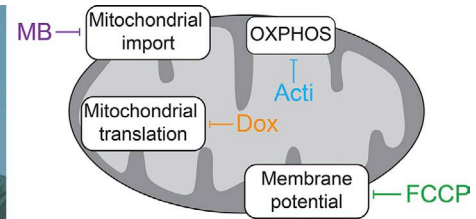
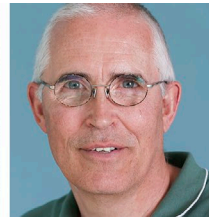
The transcription factor ATF4 coordinates the mitochondrial stress response in mammalian cells.

Mitochondria are central to cellular metabolism, generating most of the cell's ATP by oxidative phosphorylation, as well as regulating the production of numerous key metabolites. Cells must therefore quickly adapt to environmental stresses that impair mitochondrial function so that they can sustain essential metabolic pathways and limit the release of toxic reactive oxygen species from damaged mitochondria. This stress response involves coordinated changes in the expression of proteins encoded in both the nuclear and mitochondrial genomes, which are regulated by different modes of mitonuclear communication (1). Quirós et al. now take a multi-omics approach to catalog these changes in mammalian cells exposed to a variety of mitochondrial stresses and reveal a key role for the transcription factor ATF4 in vitro and in vivo (2).

One response to mitochondrial stress is the mitochondrial unfolded protein response (UPR<sup>mt</sup>), which up-regulates chaperones, proteases, and metabolic enzymes that can restore mitochondrial protein homeostasis and repair organelle function. Johan Auwerx and colleagues at the École Polytechnique Fédérale Lausanne in Switzerland have characterized the UPR<sup>mt</sup> in *Caenorhabditis elegans* (3) but the pathway's existence in mammalian cells has been harder to define. Last year, researchers reported that the mammalian UPR<sup>mt</sup> is mediated by the transcription factor ATF5 (4) but, in general, the pathway only appears to be activated under a limited set of conditions.

Auwerx and colleagues, led by postdoc Pedro M. Quirós, decided to treat HeLa cells with four molecules that stress mitochondria in different ways and measure the resulting changes in gene and protein expression by RNA sequencing and quantitative mass spectrometry (2). "It turned out that none of them—at least under the conditions we tested—induced the prototypical mitochondrial unfolded protein response," Auwerx explains. "However, they all affected cells in a major way."

Though the four different stressors had unique effects on the cells' transcriptome



**Focal Point** Pedro M. Quirós (left), Johan Auwerx (middle), and colleagues describe how mammalian cells respond to mitochondrial stress. The researchers treated cells with four drugs that disrupt mitochondrial function in different ways (right). All four stressors induced a down-regulation in mitochondrial ribosomal proteins and oxidative phosphorylation components. They also activated the cells' integrated stress response, which inhibited cytosolic protein synthesis and up-regulated the transcription factor ATF4, reducing mitochondrial function and inducing genes involved in the synthesis of amino acids and other key metabolites. The mitochondrial stress response therefore maintains cellular metabolism while reducing mitochondrial activity to limit the production of toxic reactive oxygen species. Photos courtesy of the authors.

and proteome, Quirós et al. identified many genes and proteins that were up- or down-regulated by all four molecules. "In general, we saw that proteins were down-regulated more than transcripts," Auwerx says. These down-regulated proteins included mitochondrial ribosomal proteins and components of the oxidative phosphorylation pathway. This response would therefore reduce mitochondrial activity, limiting the uncontrolled production of reactive oxygen species.

### "ATF4 puts the brakes on mitochondria."

In contrast, the four mitochondrial stressors tended to up-regulate transcripts rather than proteins. Many of these up-regulated transcripts encode proteins involved in the synthesis of serine and other amino acids. Accordingly, when Quirós et al. analyzed the metabolomes of HeLa cells treated with the mitochondrial stressors, the levels of these amino acids were increased, as were several other key metabolites, such as ceramide and certain phospholipids, that are synthesized from serine.

Many of the genes induced by mitochondrial stress contained a binding site for the transcription factor ATF4. This protein is an effector of the integrated stress response, a pathway that responds to various cellular stresses by inhibiting general protein translation while up-regulating the synthesis of

a few key transcription factors, including ATF4 (5). Deleting ATF4, or inhibiting an upstream step of the integrated stress response, blocked the induction of ATF4-target genes upon mitochondrial stress.

"We also showed that deleting ATF4 increases mitochondrial function," Auwerx says. "So, ATF4 puts the brakes on mitochondria, which is what you want to do when mitochondria are stressed."

Quirós et al. found that ATF4 was also activated by mitochondrial stress in vivo, because the transcription factor and its downstream targets were induced in mice lacking key mitochondrial proteins and in biopsies taken from patients with mitochondrial diseases. Moreover, ATF4 expression and mitochondrial function were inversely correlated even in healthy, nonstressed tissues. "So, it's not just a stress response, it's also a physiological, homeostatic pathway," Auwerx explains.

Auwerx and colleagues now want to examine the role of other transcription factors in the nuclear response to mitochondrial stress and to further investigate the mitochondrial response, which appears to be faster and largely independent of transcription.

1. Quirós, P.M., et al. 2016. *Nat. Rev. Mol. Cell Biol.* 17:213–226.
2. Quirós, P.M., et al. 2017. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201702058>
3. Houtkooper, R.H., et al. 2013. *Nature.* 497:451–457.
4. Fiorese, C.J., et al. 2016. *Curr. Biol.* 26:2037–2043.
5. Pakos-Zebrucka, K., et al. 2016. *EMBO Rep.* 17:1374–1395.

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