Peptides Signal Mitochondrial Stress

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DOI 10.1016/j.cmet.2010.02.011

The unfolded protein response (UPR^{mt}) rebalances mitochondrial protein homeostasis upon proteotoxic perturbations. Haynes et al. (2010) show that this retrograde stress signal is based on efflux of peptides derived from damaged proteins from the mitochondrial matrix to the cytosol; this initiates downstream protective responses in the nucleus to restore cellular balance.

The processing of proteins into peptides represents an ancient mechanism for protein clearance that, in vertebrates, also provides an immunological memory and antigenic history. This adaptive response distinguishes "self" from "nonself" by presentation of short peptides as antigens on the cell surface. While it is generally believed that the cellular "peptidome" derives solely from cytosolic proteins degraded by the proteasome and multiple peptidases, peptides released from mitochondrially encoded proteins can also be detected on the cell surface (Loveland et al., 1990). The origins of mitochondrial-derived peptides and their role in stress signaling has now been revealed by Haynes et al. (2010), who show that the ABC family transporter that effluxes peptides across the mitochondrial membrane also regulates the mitochondrial unfolded protein response (UPR^{mt}) and the expression of mitochondrial chaperones. This suggests a mechanism by which the degradation and clearance of damaged mitochondrial proteins initiates a mitochondrial stress signal that results in a protective response in the nucleus, which in turn enhances mitochondrial protein homeostasis (proteostasis).

Previous studies by Young et al. (2001) had established that peptides of 6–20 amino acids, derived by proteolysis of inner mitochondrial membrane proteins, could be exported through the inner membrane by the ABC transporter, Mdl1. From this point, it had been assumed that these peptides cross the mitochondrial outer membrane either through porins or the TOM complex into the cytoplasm. These observations suggested that peptide efflux serves as a means of communication between the mitochondria and their cellular environment.

Haynes et al. (2010) have now established that the efflux of mitochondrial peptides is a biologically relevant signal in the stress response. Much of our understanding of mitochondrial biogenesis centers on how nuclear-encoded cytoplasmic proteins are translocated into mitochondria, and the assembly of the electron transport chain. In a fashion similar to the UPR^{ER} that signals between the endoplasmic reticulum and the nucleus, protein damage in the mitochondria is sensed by the UPR^{mt}. Genome-wide RNAi screens using *C. elegans* have led to the identification of several regulators of UPR^{mt}, including *ubl-5*, a ubiquitin-like protein; *dve-1*, a homeobox containing transcription factor; *clpp-1*, the proteolytic component of the Clp proteases and a GTPase encoding *rheB*, *clpX1* (*K07A3.3*), *clpX2* (*D2030.2*), the Clp-ATPase subunits of the Clp protease; and *haf-1,* a putative ABC transporter and a bZIP transcription factor (*ZC376.7*) (Haynes et al., 2007, 2010). *haf-1* has sequence similarity to the yeast ABC transporter Mdl1 (Young et al., 2001).

Since in yeast the degradation of mitochondrial proteins results in an efflux of peptides by a homologous transporter (Mdl1), it was speculated that the peptide efflux by HAF-1 could also have a role in the UPR^{mt} (Haynes et al., 2010; Young et al., 2001). Indeed, mitochondria isolated from *C. elegans* exhibited an ATP-dependent release of peptides, which was abolished upon knockdown of either *clpp-1* or *haf-1*. The identification of HAF-1 sheds light on the regulation of the UPR^{mt} and suggests a signaling cascade (Figure 1) by which perturbations of the mitochondrial-folding environment leads to degradation of proteins by the matrix-localized ClpXP protease. The resulting peptides diffuse to the ABC transporter HAF-1, localized at the inner mitochondrial membrane, and are transported to the cytosol. This is thought to trigger formation of a complex between UBL-5 and DVE-1. The transcription factor complex UBL-5/DVE-1 and bZIP relocalize to the nucleus, where UBL-5/DVE-1 then activates the expression of the chaperones *hsp-6* (mt Hsp70) and *hsp-60* (mt chaperonin) and expression of *ubl-5* itself, the latter probably enhancing the signal in a feed-forward fashion. The target genes for bZIP are not yet known. Elevated levels of HSP-6 and HSP-60 translocate back into mitochondria, where they rebalance the mitochondrial proteostasis. The Ras-related GTPase RHEB is a negative regulator of the redistribution of UBL-5/DVE-1 upon mitochondrial stress and presumably has a role in the negative feedback regulation of the UPR^{mt} upon cessation of the stress condition.

The identification of the proteolytic complex (ClpXP) that generates the signaling peptides represents an important contribution to the UPR^{mt} signaling pathway and demonstrates that the efflux of peptides by HAF-1 transmits the UPR^{mt} signal from the mitochondria to the cytosol-localized transcription factors (bZIP) and UBL-5/DVE-1. These advances point toward future studies on the UPR^{mt}, to identify the signal for ClpXP specific proteolysis and establish how the released peptides transmit this signal to the downstream factors that regulate the UPR^{mt}. One could consider three scenarios (Figure 1): (I) Proteins that misfold and aggregate in the mitochondria upon proteotoxic stress could be recognized by ClpXP and subsequently degraded. This model is supported by

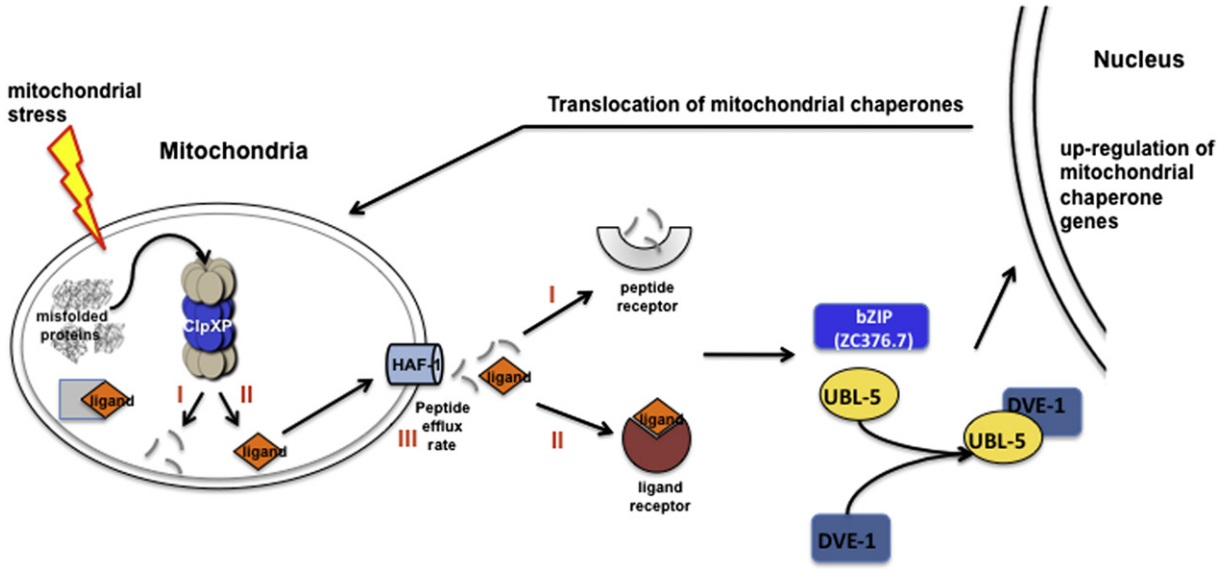

Figure 1. Mitochondrial Unfolded Stress Response (UPR^{mt}) Is Signaled by Peptides

Mitochondrial stress activates the proteolytic activity of ClpXP either by degradation of misfolded proteins into peptides (scenario I) or releasing a ligand (scenario II). The export of either the peptides or the ligand by the ABC transporter HAF-1 transmits the UPR^{mt} signal to the cytosol. It is proposed that either the recognition of the peptides or the ligand by specific receptors or the efflux rate (scenario III) is the signal to activate the transcription factors bZIP and UBL5/DVE1. Upon redistribution from the cytosol into the nucleus, the transcription factors activate the expression of mitochondrial chaperones, which subsequently translocate to the mitochondria to rebalance the mitochondrial proteostasis.

data from Haynes et al. (2010) showing that the released peptides correspond to abundant mitochondrial proteins, including metabolic enzymes and proteins of the ATP synthetase complex; (II) ClpXP is involved in both general and regulated proteolysis. Thus, a specific substrate may expose a recognition motif for ClpXP upon mitochondrial stress. For instance, ClpXP mediated proteolysis could lead to the release of a ligand, which is then transported across the mitochondrial membrane and released into the cytosol. This hypothesis is supported by the identification of a mitochondrial ABC transporter (ABCB10) that mediates transport of a heme group across the mitochondrial membrane (Shirihai et al., 2000). Both scenarios (I and II) require a specific receptor for the recognition of either peptides or a specific mitochondrial ligand to transduce the signal. A third possibility is that (III) the actual rate of efflux is the signal for the UPR^{mt}. This assumption is supported by the broad range of observed sizes and amino acid composition of the released peptides (Haynes et al., 2010).

Taken together, the work by Haynes et al. (2010) establishes that peptide efflux by an ABC transporter is utilized by eukaryotes for the regulation of a stress response pathway. Given these new insights, it is worth reflecting on the evolutionary origins of this pathway. When ancestors of contemporary eukaryotic cells formed a symbiosis with proteobacteria and subsequently incorporated them as organelles, mitochondria might have been regarded as "foreign." The peptide signaling observed here might therefore correspond to an ancient mechanism, later adopted by the cellular immune response to distinguish between "self" versus "non-self." Moreover, one might imagine this peptide signaling process evolving further into a stress response mechanism that distinguishes misfolded and damaged proteins within mitochondria. This would represent a functional expansion, of an immunological view of "self" versus "non-self," to a conformational view of folded versus unfolded, as a mechanism for monitoring the functional state of mitochondria. Stress signaling by peptides offers certain advantages, as it enables a rapid and economical response to diverse physiological challenges. Many protein and peptide signals are involved both in development, and in the regulation of physiological processes including antigen presentation as well as endocrine and insulin signaling (Boonen et al., 2009). The study by Haynes et al. adds another dimension to the signaling network of bioactive peptides.

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