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SPOTLIGHT

OMA1 clears traffic jam in TOM tunnel in mammals

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Using an engineered mitochondrial clogger, Krakowczyk et al. (https://doi.org/10.1083/jcb.202306051) identified the OMA1 protease as a critical component that eliminates import failure at the TOM translocase in mammalian cells, providing a novel quality control mechanism that is distinct from those described in yeast.

99% of mitochondrial proteins are encoded in the nuclear genome and synthesized by cytosolic ribosomes. Most mitochondrial precursors first enter the TOM translocase at the outer mitochondrial membrane (OMM) to be delivered to their final destination within the mitochondria. During import, the mitochondrial precursors must be fully unfolded in order to pass through the narrow protein-conducting pore of the import channel. However, efficient translocation of mitochondrial precursors can be disturbed by multiple problems including mitochondrial OXPHOS dysfunction, pathogenic mutations in the mitochondrial import machineries, and accumulation of disease-associated aggregation-prone proteins at mitochondrial import routes. Under these conditions, proper handling of proteins being imported often fails, resulting in the arrest of precursors in the import channel. Arrested proteins can block further import of newly synthesized precursors, leading to impaired mitochondrial function. Furthermore, these unimported mitochondrial proteins also affect general cellular protein homeostasis as they can form toxic aggregates in the cytosol. Therefore, clogged mitochondrial precursors must be eliminated to maintain mitochondrial and cellular fitness. In the last decade, several mechanisms to combat clogged mitochondrial precursors at import channels have been defined in yeast (1) (Fig. 1). However, knowledge of such mechanisms in higher organisms is limited. Krakowczyk et al. (2) uncovered a novel pathway that removes clogged mitochondrial precursors in human cells.

In yeast studies, several clogger models have been utilized to induce the arrest of mitochondrial precursors in import channels (3–5) (Fig. 1). Here, Krakowczyk et al. (2) developed a novel type of clogger. Their model clogger protein contains ATP5MG, a component of the ATP synthase, at the N-terminus, which is followed by tandem fluorescent proteins: mCherry and superfolder GFP (sfGFP). Since sfGFP is resistant to unfolding, it was expected that the attachment of sfGFP to a mitochondrial precursor would block its efficient translocation. A series of analyses showed that this engineered precursor was indeed stalled in the TOM translocase. A unique feature of this model clogger is that it did not show stable interaction with the inner mitochondrial membrane (IMM)-resident translocase, TIM. Instead, it bound to several subunits of the ATP synthase, indicating that the N-terminal portion of this clogger, ATP5MG, may be incorporated into the ATP synthase complex at the IMM. Thus, this clogging protein tethers the OMM and the IMM upon its arrest in the TOM translocase.

Presumably due to this unique feature, removal of this clogger from the translocase required a different machinery from those described in yeast. As observed in yeast clogger models, induction of this clogger impaired the import of other mitochondrial precursors, mitochondrial respiration, and cell growth. Despite these deleterious effects, this clogger was stable at the steady state. Interestingly, it was rapidly cleared upon mitochondrial depolarization. The clearance of the clogger here involved OMA1, an IMM-resident protease that is activated by mitochondrial depolarization (6). The authors set up an in vitro experimental system to track the fate of their model clogger and revealed that the clogger was released from the TOM translocase into the cytosol after the OMA1-mediated cleavage. Importantly, the artificial membrane tether known as MitoT was not cleaved by OMA1, suggesting that OMA1 may specifically recognize proteins arrested in the TOM translocase.

Most of the clearance pathways of mitochondrial cloggers described in yeast end with proteasomal degradation of the cloggers in the cytosol (3–5) (Fig. 1). This was also the case for the clogger used here in human cells. In contrast, several mechanisms of clogger recognition and extraction have been reported. In two of the pathways described in yeast, clogger extraction involves AAA-type of ATPases, either Cdc48 (human VCP/p97) or Msp1 (human ATAD1) (3, 4). However, VCP inhibition did not prevent the release of the OMA1-produced clogger fragment, suggesting that OMA1-mediated cleavage can effectively release the fragment from the TOM channel at least without VCP activity. A recent study using a different clogger model reported that ATAD1 mediates the extraction of clogged precursors

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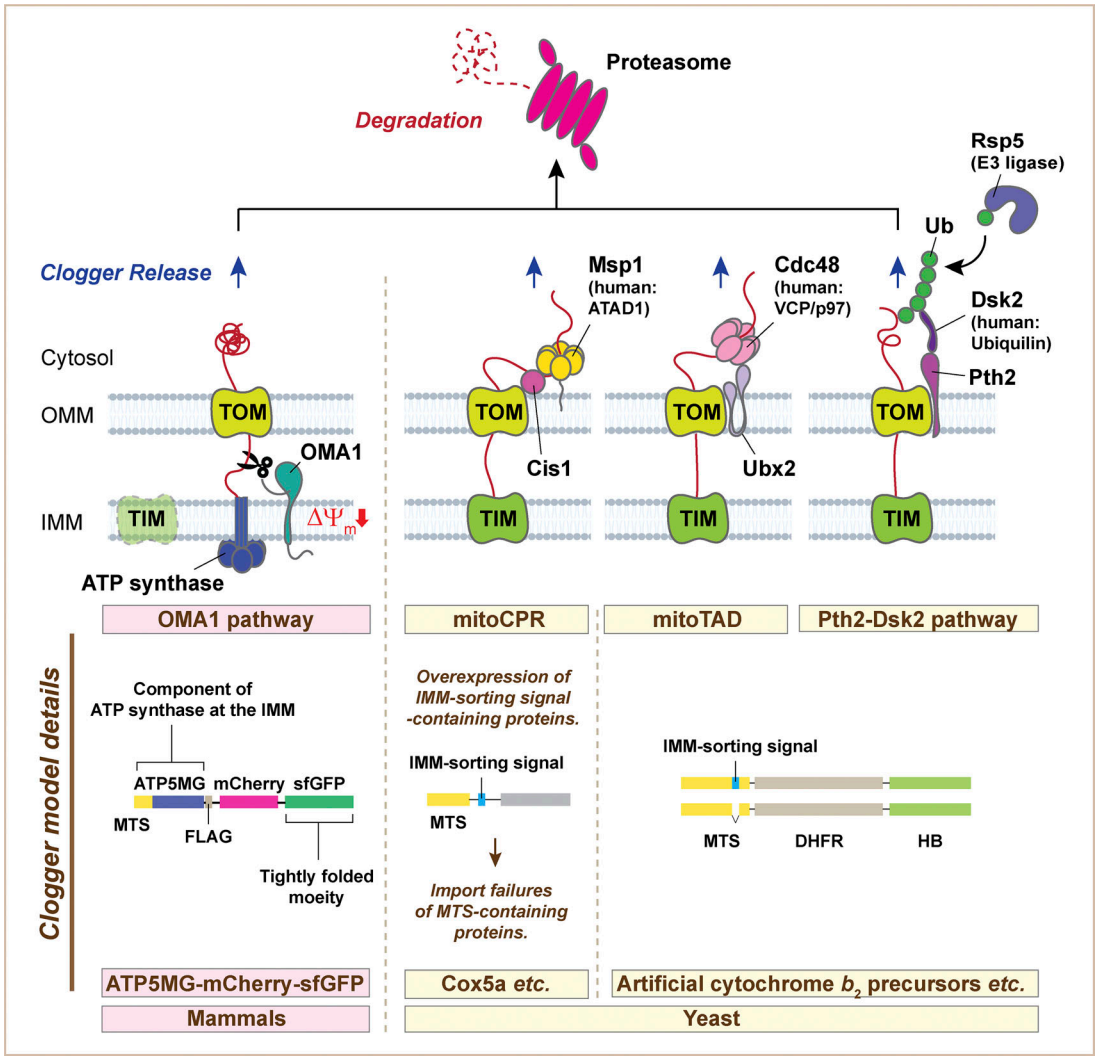

Figure 1. **Clogger removal from mitochondrial import channels in mammals and yeast.** Molecules involved in clogger removal and representative cloggers utilized in each pathway are shown. The mammalian version of clogger developed by Krakowczyk et al. (2) is integrated in the IMM component, ATP synthase, upon clogging at the TOM translocase. In this pathway, the clogger is first cleaved by OMA1 upon mitochondrial depolarization, which promotes the clogger release from the TOM translocase. In all reported pathways, cloggers are degraded by proteasome. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; MTS, mitochondrial targeting sequence; DHFR, dihydrofolate reductase; HB, haem-binding domain.

in mammalian cells (7, *Preprint*). It remains unclear whether ATAD1 is generally involved in the release of cleaved import intermediates from the TOM translocase.

The authors demonstrated the importance of this new clogger clearance pathway by monitoring mitochondrial cristae structure. Cristae structure was severely damaged by induction of their model clogger. However, when OMA1 was stimulated by mitochondrial depolarization, cristae structure was partially but significantly restored, suggesting that the OMA1-mediated clogger clearance can reverse the clogging-induced damage.

This study provides a novel insight into the mammalian quality control pathway that resolves mitochondrial import failure. However, several questions remain. For example, although the unique clogging model uncovered a novel clogger clearance mechanism, the actual physiological contexts in which this type of import arrest occurs remain unclear. In the pathway identified, mitochondrial depolarization was critical for the OMA1-mediated clogger clearance. Paradoxically, mitochondrial uncoupling is a known inducer of general import failure. Nevertheless, several possibilities can be considered. It is known that OMA1 activation by mitochondrial depolarization is a very rapid process occurring within 5 min (6). Therefore, in physiological contexts, even local, transient activation of OMA1 may be sufficient to prevent the spread of pernicious import defects before the entire mitochondrial network is damaged. Some specific disease conditions may also be relevant. A previous study showed that some types of Parkinson's disease-related PINK1 mutants are cleared from mitochondria by mechanisms similar to those described here (8). Therefore, these pathogenic PINK1 mutants may be one of the substrates of the pathway identified. It is also noteworthy that the OMA1-mediated cleavage of arrested mitochondrial proteins can trigger stress signaling, as exemplified by the cleavage of DELE1, which activates the integrated stress response (9). Recent work on this pathway

has identified a ubiquitin E3 ligase complex for proteasomal degradation of unimported mitochondrial precursors (10). However, an E3 ligase that recognizes the OMA1-produced clogger fragment has not been defined. Does a common E3 ligase eliminate different types of import problems? Or are there any specialized E3 ligases dedicated to the clearance of clogged precursors? Such questions should be answered. In yeast, mitochondrial import problems activate not only local aids at the TOM translocase, but also systemic aids that involve transcriptional and translational responses (1). Therefore, further delineation of these possibilities would be also important in this newly developed clogging model. As such, the results presented here will open a new direction for research on mitochondrial quality control in mammals.

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