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An unconventional pathway for mitochondrial protein degradation

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ABSTRACT

Many vital metabolic pathways take place in mitochondria, but some of the associated processes generate toxic substances including reactive oxygen species that can damage proteins and DNA. Therefore, it is critical to maintain normally functioning mitochondria to achieve proper cellular homeostasis. Along these lines, mitochondrial dysfunction is associated with numerous diseases, and mitochondria quality control is essential for cell survival. The maintenance of functioning mitochondria is particularly important in aging cells, and there is a strong relationship between cellular aging and dysfunctional mitochondria. The best characterized pathway that is responsible for the elimination of damaged mitochondria is mitophagy, a selective type of autophagy. In yeast, mitophagy requires the mitochondrial protein Atg32 to serve as a receptor for recognition and sequestration by a phagophore. Although conventional mitophagy has been extensively studied, recent research suggests that an unconventional pathway, which is independent of Atg32, contributes to the removal of mitochondria.

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In the study of Hughes et al.,[1] the authors were first interested in mitochondrial protein degradation in aged cells; in yeast age can be determined by the presence of bud scars. They found Tom70-GFP, a mitochondrial outer membrane protein tagged with the green fluorescent protein, is delivered to the vacuole only in aged cells. They further showed that this delivery is independent of Atg32, and still occurs in *atg32Δ* cells. Nonetheless, the general autophagy machinery and mitochondria fission machinery, which are both involved in canonical mitophagy,[2-4] are still required in this delivery process; *atg5Δ and dnm1Δ* mutant strains are defective for Tom70-GFP vacuolar delivery.

Next, the authors asked what triggered this mitochondrial degradation pathway in aged cells. Previous studies with aged cells indicated that vacuole dysfunction is associated with mitochondria disruption. Thus, Hughes et al. tested whether loss of vacuole acidity leads to degradation of mitochondrial proteins. Treatment with concanamycin A, which blocks the function of the vacuolar-type H^+-translocating ATPase, of young cells results in a reduction of mitochondria membrane potential and an accumulation of Tom70-GFP in the vacuole, mimicking the phenotype seen in aged cells. This finding suggested that the loss of vacuolar acidity results in activation of the mitochondria protein degradation pathway. Because mitochondria membrane potential was reduced after loss of vacuolar acidity, the authors further investigated whether chemically-induced loss of mitochondria membrane potential leads to the degradation of Tom70; however, this does not appear to be the case. Similarly, oxidative stress, in the form of hydrogen peroxide, fails to induce the degradation of mitochondria. Thus, the loss of vacuole acidity triggers Tom70 degradation through an unknown mechanism.

During the analysis of Tom70-GFP delivery, the authors observed that Tom70-GFP forms a distinct vesicle-like structure before being sent to the vacuole. They termed this structure a 'mitochondrial-derived compartment (MDC)'. The MDC does not appear to be a standard autophagosome because it can still be formed in *atg5Δ* cells; however, MDC cannot enter the vacuole in cells lacking Atg5, indicating that the autophagy machinery is important for MDC delivery to the vacuole but not its formation. Similarly, MDC formation is not affected in *dnm1Δ* cells. Conversely, MDC stays near the mitochondria in this mutant, suggesting that Dnm1 is important for MDC release from the mitochondria.

Having discovered this novel structure, the authors tried to identify the protein components present in the MDC. They expressed Tom70-mCherry in a strain expressing proteins of interest tagged with GFP, and looked for proteins that colocalized with Tom70-mCherry—as a marker of the MDC—after treatment with concanamycin A. Among 469 mitochondrial proteins examined, 26 localize to the MDC and are sent to the vacuole for degradation. Tom70 plays a role in one of the mitochondrial protein import pathways. Interestingly, all proteins that rely on the Tom70 import pathway are present in the MDC. Conversely, proteins that rely on other mitochondrial import pathways are excluded from the MDC. Moreover, through the use of a recombination-induced tag exchange system,[5] the authors showed that the MDC contains both newly synthesized and preexisting proteins. Taken together, these

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results suggest that the MDC is cargo selective and mainly contains proteins that bind to Tom70.

Finally the authors investigated the requirement for MDC formation. Because Tom70 plays a central role in this unconventional pathway, the authors focused on this protein and its paralog, Tom71. In this case they tagged Cox7, another component of the MDC, with GFP; normal MDC formation can therefore be detected by visualizing Cox7-GFP puncta instead of Tom70-GFP. In a *tom70Δ* mutant the Cox7-GFP signal is absent in the vacuole and MDC formation is severely blocked, whereas a *tom70Δ tom71Δ* double knockout strain displays essentially a complete block in formation of the MDC. Collectively, these results indicate that Tom70 and Tom71 are required for MDC formation.

Overall, the authors discovered an unconventional pathway for degradation of mitochondria proteins. Unlike the canonical degradation that is used to degrade large portions of mitochondria during mitophagy, only a subset of mitochondrial proteins is broken down in this newly discovered pathway. The presence of this second degradative mechanism indicates that mitochondria homeostasis is under complex regulation. While the study enlarges our knowledge regarding mitochondria quality control, further questions remain to be answered. For example, it will be interesting to know the detailed signaling pathway that triggers MDC formation. Also, the relationship between MDC delivery and the canonical autophagy machinery needs to be further studied. Ultimately, the relationship between this pathway and age-related diseases may be a very interesting topic.

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No potential conflicts of interest were disclosed.

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