Organelle Turnover

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Organelle turnover: a USP30 safety catch restrains the trigger for mitophagy and pexophagy

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Summary

It is crucial to remove dysfunctional mitochondria and peroxisomes to prevent cellular damage. Recent work suggests that under basal conditions USP30, a deubiquitinating enzyme, works to ensure that both these organelles are only turned over at the right time.

Main Text

Mitophagy, the autophagic delivery of mitochondria to lysosomes, is largely seen as a protective mechanism whereby dysfunctional mitochondria, which can release harmful reactive oxygen species (ROS), are degraded to prevent cellular damage [1, 2]. Mitophagy came to the centre stage when landmark studies showed that PINK1, a mitochondrial protein kinase, and Parkin, an E3 ubiquitin ligase, operate sequentially to tag mitochondria with a ubiquitin “eat-me” signal to enable specific mitochondrial engulfment by forming autophagosomes [3-5]. As PINK1 and Parkin can be mutated in hereditary Parkinson’s disease, this suggests that impaired mitophagy may play a disease role and that targeting it could prove therapeutically beneficial [6]. Ways to do this received a boost when it was shown that USP30, a mitochondrial deubiquitinase (DUB), could inhibit this pathway [7]: If the Parkin-derived mitochondrial ubiquitylation is the “on-switch” for mitophagy, then deubiquitylation will be the “off-switch”. Thus, inhibiting USP30 should enhance mitophagy and help clear dysfunctional mitochondria. Consequently, a deeper understanding of USP30 regulation coupled with ways to target this protein is high on the research agenda. Recent work from Marcassa et al. [8] has delved more into the function of USP30 and revealed that even though USP30 regulates mitophagy, it may do so in an unexpected manner.

Multiple mechanisms of mitophagy are emerging [2], but it is the aforementioned PINK1/Parkin-dependent pathway that has received the most attention. A significant body work (reviewed by Harper et al. [9]) has led to the following model. Upon mitochondrial depolarisation, PINK1 becomes stabilised on the outer mitochondrial membrane (OMM) and can then phosphorylate both ubiquitin and Parkin. Both these phosphorylation events are required for full Parkin activation, which then goes on to ubiquitylate multiple OMM substrates. The generated ubiquitin chains are further phosphorylated by PINK1, resulting in a feed-forward enhancement of Parkin activity and robust OMM ubiquitylation that in-turn acts as the “eat-me” signal for the autophagosome engulfment (Fig. 1A). It is at this ubiquitylation step that USP30 was first implicated as a negative regulator of mitophagy, with its overexpression or loss inhibiting or enhancing mitophagy respectively [7].

There has been some debate however on the significance of the PINK1/Parkin pathway as the majority of experiments carried out have relied on cells overexpressing large amounts of Parkin coupled with harsh chemical mitochondrial depolarising agents. While this results in a striking and almost complete loss of mitochondria, which can easily be detected, it is hard to envision when and where such extreme scenarios might arise *in vivo*. Regardless, these experiments have been instrumental in elucidating the mechanisms of Parkin-driven

mitophagy, with the caveat that overexpression artefacts could have clouded some data interpretation. The recent advent of more sensitive fluorescent probes has now allowed reliable assessment of mitophagy under more physiological conditions [10, 11]. Critically, these reporters have been used to generate mouse models that show mitophagy is indeed a physiological and pervasive process in mammals, making it reasonable to assume that its disruption will have impacts on cellular health and disease [12, 13]. Surprisingly though, loss of the PINK1/Parkin pathway in mice fails to have any notable difference on mitophagy under normal conditions [14], a fact that is also supported by a related study in flies [15]. While these findings highlight that the PINK1-Parkin pathway is not the major route for mitophagy *in vivo*, they do not mean it is not an important one. PINK1-dependent mitophagy may be a rare event, or only become relevant under distinct stress conditions. Thus, the search for the physiological significance of the PINK1/Parkin pathway is still ongoing.

Given the above, Marcassa et al. investigated the cellular role of USP30 without Parkin overexpression and in the absence of acute chemical depolarisation [8]. Using the previously mentioned sensitive mitophagy reporter systems, the authors showed that the cellular loss of USP30 resulted in increased mitophagy, implying that ubiquitylation is indeed a key mitophagy signal. The immediate assumption that this was mediated by PINK1/Parkin activation did not hold true though, as loss of PINK1 alone in this system did not reduce basal mitophagy. However, when the authors examined the loss of USP30 in the background of no PINK1, they found that the previously observed mitophagy increase was abolished. This could mean that loss of USP30 itself results in mitochondrial stress that activates the PINK1 pathway, or that PINK1 does play a role in basal mitophagy. The authors favour this latter scenario and have proposed that USP30 acts upstream of PINK1, rather than on the ubiquitin chains produced following activation of Parkin. The data support this hypothesis if the current model for Parkin activation holds true, in that it requires phosphorylation of pre-existing ubiquitin as well as that of Parkin itself. Thus, USP30 could be acting on the low level of pre-existing mitochondrial ubiquitylation and in this way prevents “tonic” activation of mitophagy: in essence it acts a safety catch to ensure mitophagy is not accidentally triggered under normal conditions (Fig. 1A).

Like mitochondria, peroxisomes are also oxidative in nature due to fatty acid beta-oxidation and detoxification of hydrogen peroxide [16]. Hence the autophagy of peroxisomes, termed pexophagy, is thought to perform a similar role to mitophagy in that it can help eliminate dysfunctional or excess peroxisomes to limit the production of damaging ROS. The similarities do not end here as the basic mechanism of ubiquitylation as an autophagosome-engulfment signal also appears to be conserved. Indeed, ectopic expression of peroxisomal proteins conjugated to ubiquitin is sufficient to trigger their delivery to lysosomes [17]. Unlike mitochondria though, PINK1 and Parkin are not thought to play a role in pexophagy, with the peroxisomal E3 ligase PEX2 implicated instead [18, 19]. If ubiquitylation is the key eat-me signal for peroxisomes then surely an “off-switch” or DUB will also be important in its regulation? The identity of such a DUB was not known but in the same USP30 study, Marcassa and colleagues noted that USP30 also localised to peroxisomes and its depletion also resulted in increased pexophagy (Fig. 1B). Peroxisomes though, have much closer relationship with mitochondria than just their common oxidative nature would suggest. Extensive contact sites exist between these organelles and recently mitochondria were shown to be involved in peroxisome generation [20]. Thus, it is possible that the observed

increase in pexophagy upon loss of USP30 is an indirect effect of peroxisomes being in close association with mitochondria during mitophagy. Marcassa et al., took two approaches to help rule this out. Firstly, they were able to show that the peroxisomal and mitochondrial targeting of USP30 required distinct sequences around USP30's transmembrane domain. Secondly, the authors used a neat trick to rid the cells of mitochondria. By taking advantage of the fact that Parkin overexpression, coupled with mitochondrial depolarisation, results in cellular depletion of this organelle, they were able to show that USP30 can still localise to peroxisomes independently of mitochondria. Thus, USP30 does appear to be a *bone fide* DUB regulating pexophagy as well as mitophagy.

The conservation of USP30 mechanism in regulating both mitochondrial and peroxisomal autophagy pathways suggests that the turnover of these organelles could be co-ordinated under certain conditions. How this might proceed is currently unknown, as are the finer details of USP30 mechanism. What are the E3 ligases it opposes? For mitophagy, if it acts upstream of PINK1, this implies a ligase other than Parkin. In the case of peroxisomes, PEX2 is a candidate but evidence for a USP30 link is lacking. Related, the actual ubiquitylated substrates are not clear as are the ubiquitin chain linkage types. Finally, USP30 has been proposed as a promising mitophagy-enhancing drug target. While the Marcassa study still supports such a role for USP30, it suggests that healthy mitochondria could be removed through enhancement of basal mitophagy and this may be less beneficial. Secondly, inhibiting USP30 will also result in pexophagy, which might have consequences in tissues such as liver and kidney where these organelles play an important role in detoxification. More work is obviously needed, but our knowledge of how organelles are turned over, and the consequences of this, is growing rapidly. Certainly, the complex interplay between ubiquitylation and deubiquitylation in these specific autophagy pathways is providing plenty of food for thought.

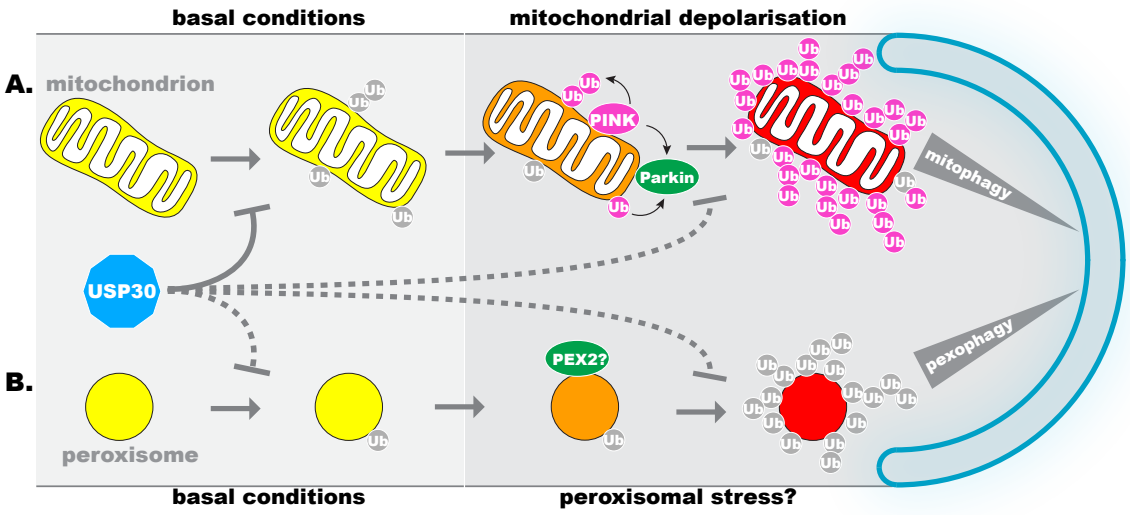

Figure 1. **USP30 reverses mitochondrial and peroxisomal ubiquitylation to prevent their autophagy**. (**A**) USP30 removes the low level of mitochondrial ubiquitylation that occurs under basal conditions. This prevents activation of Parkin, excessive ubiquitylation and mitophagy should PINK1 become stabilised without robust mitochondrial depolarisation. (**B**) USP30 also limits pexophagy through peroxisome deubiquitylation, though the exact stage that USP30 acts here requires confirmation.

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