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A mammalian pexophagy target

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Abstract

Protein ubiquitination in mammals is known to trigger selective autophagy of peroxisomes, through a process termed pexophagy. Until now, the peroxisomal target for pexophagy-related ubiquitination was controversial, but two studies have identified the protein PEX5 as the real candidate.

In addition to roles in protein trafficking and turnover, protein ubiquitination in mammals often initiates selective autophagy of protein aggregates (aggrephagy), bacteria (xenophagy), mitochondria (mitophagy) or peroxisomes (pexophagy)¹. In yeast models, four peroxisome membrane-associated proteins, Pex5 (also called the PTS1 receptor), Pex7 (PTS2 receptor), Pex20 (PTS2 co-receptor) and the ubiquitin-conjugating enzyme, Pex4, are ubiquitinated during the peroxisomal matrix protein import cycle, and could constitute targets that engage the autophagy machinery². Of these, only PEX5 is known to be ubiquitinated in mammals³. Given that ubiquitin (Ub) is known to target cargoes for selective autophagy¹, its putative role in mediating pexophagy was initially revealed by the use of artificial, ectopic constructs expressing Ub fused to the cytosolic domains of two peroxisomal integral membrane proteins, PEX3 and PMP34. These fusion proteins triggered pexophagy by engaging ubiquitin-binding autophagy adapters (p62, NBR1) and the core autophagic machinery⁴, but the possibility remained that these fusions might represent non-physiological circumstances. Subsequently, over-expressed PEX3 was reported to be ubiquitinated and to drive pexophagy⁵. However, its overexpression and the fact that pexophagy still occurred when PEX3 ubiquitination was blocked, cast some doubt on the biological relevance of the findings and suggested the possibility of other relevant targets. Thus the identification of a physiological link between mammalian ubiquitination and pexophagy would constitute a significant advance in the field. Two related studies, by Zhang et al.⁶ (published in this issue) and Nordgren et al.⁷ now identify PEX5 as the physiological ubiquitination target that promotes pexophagy.

Zhang et al.⁶ show that reactive oxygen species (ROS) activate a signaling cascade that targets peroxisome-associated PEX5, through the action of the Ataxia-telangiectasia mutated (ATM) kinase. ATM kinase is shown to phosphorylate PEX5, thereby activating a nearby site for ubiquitination, which tags peroxisomes for selective pexophagy. The study uncovers a credible, physiological target for pexophagy driven by ROS signaling.

The ATM kinase, a nuclear, DNA-damage sensor, was previously reported to be also associated with peroxisomes⁶. In the present work, ATM was shown to reside on the cytosolic face of the peroxisome membrane, a localization that was stimulated by H₂O₂ treatment. No peroxisomal localization of ATM kinase was apparent in fibroblasts from peroxisome-deficient Zellweger syndrome patients, which are defective in *PEX6*, a gene required for peroxisome biogenesis. Moreover, ATM localization to peroxisomes was dependent on PEX5 and ATM, which is activated most likely by locally-generated ROS. The authors showed that PEX5, which generally recognizes only C-terminal, conserved variants of a tripeptide sequence, PTS1 (peroxisomal targeting signal 1)², surprisingly interacted with the tripeptide SRL, an internal PTS1-like sequence in ATM. Mutation of SRL affected ATM peroxisomal localization, but not ATM kinase activation by ROS or the role of ATM in sensing DNA damage. ATM was demonstrated to phosphorylate human PEX5 at Ser141, a modification that was necessary for the subsequent mono-ubiquitination of PEX5 at K209 (Fig. 1). PEX5(S141A) and PEX(K209R) mutants, that could not be phosphorylated and ubiquitinated, respectively, were deficient in ROS-induced pexophagy, reflecting the importance of these modifications for this process. Although not explicitly studied, these PEX5 modifications occur presumably on both the short and long isoforms of the protein, which result from alternative splicing of its mRNA⁸. Pexophagy is also dependent on another adapter, NBR1⁹, which was not tested. Peroxisome-associated RING E3-ubiquitin ligases are known to be required for PEX5 ubiquitination³, and their depletion reduced PEX5 monoubiquitination.

Peroxisomes are involved in oxidative reactions, such as the β -oxidation of branched and very long chain fatty acids, and produce ROS, especially H₂O₂. ROS-mediated ATM kinase signaling is known to inhibit the mammalian target of rapamycin complex 1 (mTORC1) and to activate the unc51-like protein kinase 1 (ULK1) to stimulate autophagy¹⁰. Thus, the authors confirmed that peroxisome-generated ROS activated ATM, leading to inhibition of mTORC1 and activation of both ULK1 and selective autophagy (Fig. 2). They also demonstrated that ubiquitinated PEX5 recruited the autophagy adapter protein, p62⁴, engaging the autophagic core machinery and autophagosomes, with peroxisomes, to induce pexophagy (Fig. 2). Notably, the dual action of ATM kinase in targeting peroxisomes for pexophagy through ubiquitination, and the simultaneous activation of the core autophagy machinery (ULK1) is necessary for pexophagy to occur (Fig. 2).

PEX5 is predominantly cytosolic with only a fraction residing transiently at the peroxisome membrane. If PEX5 associates with ATM kinase in the cytosol and recruits this kinase to the peroxisome membrane, what prevents ATM kinase from activating the phosphorylation and degradation of PEX5 prematurely, without involving the peroxisome? One possibility is that ATM kinase is only activated, through ROS-mediated dimerization, on peroxisomes, whereas another may be that the E3 ubiquitin ligases (PEX2, PEX10 and PEX12), which are required for PEX5 ubiquitination are themselves located on peroxisomes. However, one should be cautious about the conclusion that the peroxisome-associated RING E3 ubiquitin ligases are responsible for the mono-ubiquitination at K209, because these E3 ligases are also involved in other ubiquitination reactions on PEX5⁷. The loss of monoubiquitination observed here, presumably at K209, following the downregulation of these E3 ligases, could

be a consequence of the fact that PEX5 is not in the right stage, or location, in the matrix protein import cycle, for this modification to occur.

During the matrix protein import cycle, PEX5 undergoes two other types of ubiquitination (Fig. 1) – a mono-ubiquitination on Cys11 that is necessary for its recycling from the peroxisome membrane back to the cytosol for another round of import¹¹, or poly-ubiquitination of lysine/s that target/s PEX5 for extraction from the peroxisome membrane and degradation by the ubiquitin-proteasome system (UPS)¹². This naturally raises the question as to why ubiquitination at these residues in PEX5 does not also target it for pexophagy. Indeed this can happen, as shown by the demonstration that the N-terminal mono-ubiquitination of PEX5 at Cys11 induces pexophagy in SV40 T antigen-transformed mouse embryonic fibroblasts⁷. These authors found that PEX5 fused to a bulky C-terminal tag was delivered to peroxisomes and mono-ubiquitinated at Cys11, but its failure to be exported from peroxisomes triggered pexophagy (Fig. 2), and suggested a model wherein it is the mono-ubiquitination of peroxisome-associated PEX5 at Cys11 that drives pexophagy. This study does not report ubiquitination at K209; however their system did not involve pexophagy induction by ROS, but rather a process driven by providing the PEX5 recycling machinery with an artificial PEX5 fusion protein that might activate pexophagy by a different route. Also, although p62 played a key role in pexophagy mediated by PEX5 K209 ubiquitination, the adapter protein/s that recognize/s Cys11 ubiquitination to target peroxisomes for pexophagy in SV40-transformed cells was not identified (Fig. 2). Additionally, the cells used in the two studies are different and there is evidence of cell type-specific, as well as cell immortalization-dependent, variations in pexophagy⁷. An unanswered question from the two studies^{6, 7} is whether the mechanisms they describe are true for pexophagy activated by different signaling mechanisms or specific to certain cell types.

These contrasting reports that ubiquitination on two different residues on PEX5 (Cys11 versus K209) activate pexophagy, also beg the question of whether these are unrelated or interdependent mechanisms. It is unclear, for example, whether the PEX5(K209R) mutant is trapped on peroxisomes during the matrix protein import cycle, and if this is the underlying reason for triggering pexophagy. It is important to note that PEX5 is not a passive bystander to the effects of ROS. Cysteines located near the N-terminus of yeast and mammalian PEX5 proteins are mono-ubiquitinated, and are also sensitive to redox conditions^{11, 13}, which affect the oligomeric status of yeast Pex5 through disulfide bonding, as well as its affinity for cargo¹³.

These studies, while whetting our appetites, raise questions that are ripe for further investigation. Is PEX5 the only ubiquitination target driving pexophagy? There are hints that this might not be the case⁶. Does PEX5 fulfill this function primarily in response to ROS, or also to other stimuli, such as hypoxia¹⁴ (Fig. 2), and damaged or superfluous peroxisomes? Is pexophagy triggered only by peroxisome-generated ROS, or also by other cellular mechanisms that activate ROS¹⁵? PEX5 is a pivotal protein confronted with three possible fates during the matrix protein import cycle. Should it be recycled to allow another round of import of matrix proteins, or degraded by the UPS when its export from the peroxisome to the cytosol is inhibited², or alternatively destroyed through pexophagy along with the

organelle it is associated with^{6, 7}? How is the choice between these fates determined mechanistically? Could pexophagy be the mammalian substitute for clearing jammed PTS receptors, instead of the UPS pathway found in yeast² but not yet in mammals? By identifying PEX5 as a mammalian target for ubiquitination-induced pexophagy, the studies of Zhang et al. and Nordgren et al. represent valuable launching pads for future research into these open questions.

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Fig. 1.

Protein modifications on *H. sapiens* PEX5L (long isoform) that regulate pexophagy. The 639 amino acid PEX5L protein has an N-terminal domain responsible for receptor dynamics and a C-terminal TPR domain responsible for PTS1-cargo and ATM kinase binding. Cys11 mono-ubiquitination by members of the E2D/UbcH5 family³ drives receptor recycling from peroxisomes to the cytosol, after intra-peroxisomal cargo release, during the matrix protein import cycle. When receptor recycling is prevented by fusion of a bulky protein (GFP) to the C-terminus, mono-ubiquitination at Cys11 becomes necessary for pexophagy in SV40 T-antigen transformed MEFs⁷. PEX5L associates with ATM kinase and docks at the cytosolic face of the peroxisome membrane. Following ROS signaling, particularly from peroxisomes, ATM is likely to be activated by dimerization and phosphorylates Ser141. This modification then activates an unknown ubiquitin-conjugating enzyme, which mono-ubiquitinates K209. Mono-ubiquitination at K209 recruits p62, which partners with the core autophagy machinery to trigger pexophagy⁶.

Fig. 2.

Signaling of pexophagy by ROS⁶, hypoxia¹⁴ and inhibition of PEX5 export from peroxisomes⁷. Pexophagy depends on (a) a peroxisomal target that tags the organelle for turnover (e.g. PEX5); (b) activation of the core autophagy machinery and (c) ubiquitin-binding pexophagy adapters, such as NBR1 and p62^{4, 9}. All these components were studied in the ROS signaling pathway, but the role of NBR1 was not investigated⁶. It is likely, but not definitive, that ATM is activated by ROS at peroxisomes. The pexophagy pathway studied by Nordgren et al.⁷, which identifies PEX5 as the peroxisomal target, appears to be independent of the known pexophagy adapters, p62 and NBR1, and leaves unclear how the core autophagy machinery is activated, although this machinery is involved⁷. In hypoxia-induced pexophagy, neither the peroxisomal receptor, nor how the core autophagy machinery is activated, was studied, although this machinery is necessary. Both NBR1 and p62 are activated and relocate to the peroxisomes during hypoxia-induced pexophagy¹⁴. Questionmarks denote open questions and undetermined points.