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Atg36

The *Saccharomyces cerevisiae* receptor for pexophagy

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Eukaryotic cells adapt their organelle composition and abundance according to environmental conditions. Analysis of the peroxisomal membrane protein Pex3 has revealed that this protein plays a crucial role in peroxisome maintenance as it is required for peroxisome formation, segregation and breakdown. Although its function in peroxisome formation and segregation was known to involve its recruitment to the peroxisomal membrane of factors specific for these processes, the role of Pex3 in peroxisome breakdown was unclear until our recent identification of Atg36 as a novel *Saccharomyces cerevisiae* Pex3-interacting protein. Atg36 is recruited to peroxisomes by Pex3 and is required specifically for pexophagy. Atg36 is distinct from Atg30, the pexophagy receptor identified in *Pichia pastoris*. Atg36 interacts with Atg11 in vivo, and to a lesser extent with Atg8. These latter proteins link autophagic cargo receptors to the core autophagy machinery. Like other autophagic cargo receptors, Atg36 is a suicide receptor and is broken down in the vacuole together with its cargo. Unlike other cargo receptors, the interaction between Atg36 and Atg8 does not seem to be direct. Our recent findings suggest that Atg36 is a novel pexophagy receptor that may target peroxisomes for degradation via a noncanonical mechanism.

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Peroxisomes are dynamic organelles present in almost all eukaryotic cells. Their abundance is responsive to the metabolic requirements of the cell. Methylotrophic yeasts grown on fatty acids or methanol extensively proliferate these organelles and when switched to conditions where they are no longer required, peroxisomes are broken down via autophagy. The selective degradation of peroxisomes requires the core autophagic machinery and Atg11. The peroxisomal membrane proteins Pex3 and Pex14 have been implicated in pexophagy, but their roles remain unclear because deletion of the corresponding genes results in a total absence of, or the generation of malformed, peroxisomes, respectively. Using *S. cerevisiae* as our model system we observed pexophagy under conditions of postlogarithmic respiratory growth, and after proliferation of peroxisomes followed by nitrogen starvation. To understand the role of Pex3 in pexophagy, we used two approaches. First, we isolated new *pex3* alleles that were affected specifically in pexophagy, but were still functional in peroxisome formation and segregation. Second, we identified a novel Pex3-interacting protein using the yeast two-hybrid system. We found that this protein is not required for peroxisome formation, function or segregation. Instead we found it to be required for pexophagy but not for bulk autophagy or other forms of selective autophagy including mitophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway. We called this new protein Atg36.

Atg36 and Pex3 bind each other in vitro and as yeast two-hybrid constructs. Atg36 localizes to peroxisomes of wild-type cells, but is present in the cytosol of cells lacking Pex3 as well as in cells containing the pexophagy-deficient *pex3* allele. Furthermore, relocalization of Pex3 to the mitochondrial outer membrane

results in Atg36 also localizing there. These observations show Pex3 is required for recruitment of Atg36 to peroxisomes and that this recruitment is required for pexophagy. Interestingly, mitochondrial-redirected Pex3 (and thus Atg36) can complement the mitophagy defect of cells lacking the mitophagy receptor Atg32. This suggests that Pex3 and Atg36 form part of the recognition module, i.e., the receptor for autophagic degradation of peroxisomes.

We hypothesize that activation of pexophagy is a two-step process. In the first step we see an increase in peroxisome-bound Atg36 levels under peroxisome proliferation conditions, but pexophagy occurs only after the second step when cells are switched to nitrogen starvation conditions and Atg36 is modified. This is reminiscent of, for instance, the phosphorylation-dependent activity of the selective autophagy receptors Atg30, Atg32 and OPTN/optineurin. Such a two-step mechanism would allow for a rapid response to a change in environmental conditions.

Studies on other substrates for selective autophagy in *S. cerevisiae* have revealed that cargo receptors interact with Atg8 and Atg11. Using co-immunoprecipitation experiments we show that Atg36 interacts with Atg11 and to a lesser extent with Atg8. Higher levels of Atg11 and Atg8 are found in complex with Atg36 under pexophagy conditions. Furthermore, we observed close proximity of peroxisomes with Atg11 in cells where the progression of autophagosome formation is blocked. Like other receptors for selective autophagy, Atg36 remains associated with peroxisomes during autophagosome formation and is broken down together with its substrate in the vacuole. Furthermore, overexpression of Atg36 accelerates pexophagy under pexophagy-inducing conditions. All these characteristics support a model whereby Atg36 acts as pexophagy receptor.

Selective autophagy receptors in mammalian cells and in yeast all interact directly with Atg8/LC3 via a conserved Atg8-interacting motif (AIM). Atg36 contains eight putative AIMs and mutagenesis of these did not identify a single motif that is required for function. Furthermore, we were unable to detect a direct interaction in vitro or a yeast two-hybrid interaction between Atg36 and Atg8. This suggests that Atg36 interacts indirectly with Atg8 and this may explain why only very low levels of Atg8 coimmunoprecipitate with Atg36. Alternatively, multiple AIMs may function in Atg36 and for technical reasons we have been unable to detect an interaction between Atg8 and Atg36 in vitro.

Atg8 performs multiple functions including recognition of selective autophagy receptors. However, the requirement for direct receptor recognition seems to differ between organisms and receptors. Thus far, interaction with Atg8/LC3 has been shown to be crucial for the function of mammalian receptor proteins as well as for the *S. cerevisiae* Cvt pathway receptors Atg19 and Atg34. In contrast, disruption of the AIM in the mitophagy receptor Atg32 results in only a minor reduction of mitophagy, and disruption of the AIM binding site in Atg8 reduces mitophagy to 50%. This suggests that the receptors for some selective forms of autophagy may not require direct interaction with Atg8.

Our work also opens the way for studying how cells regulate peroxisome abundance using a single anchoring site (Pex3) for factors required for peroxisome formation, segregation and turnover. Further studies on the regulation of these processes will provide us with new insights into organelle dynamics in response to intracellular and environmental cues.

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