

SPOTLIGHT

Atg9 is a conserved regulator of lysosome repair

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The ATG9 transmembrane protein scrambles lipids to regulate phagophore formation during autophagy. Two recent studies from Peng et al. (<https://doi.org/10.1083/jcb.202411092>) and De Tito et al. (*Dev. Cell.* <https://doi.org/10.1016/j.devcel.2025.05.007>) identify ATG9 as a conserved regulator of lysosome repair in *Caenorhabditis elegans* and human cells, but differences in repair mechanisms exist between these taxa.

Lysosomes are the key catabolic organelles that receive autophagic cargos from multiple sources, such as proteins, pathogens, and other organelles, for clearance (1). The integrity of lysosomes safeguards cell homeostasis and health. Lysosomes contain proton pumps to maintain a low pH and multiple hydrolases that are compatible with the acidic environment (2). Lysosomal hydrolases are the enzymes responsible for digesting cellular cargoes, including lipids, nucleic acids, proteins, and organelles. Lysosomal membrane rupture is caused by surfactant-containing substances, internalized infectious pathogens, neurotoxic aggregates, and silica crystals (3). Surfactant integration into lysosomal membranes and membrane lipid peroxidation remodels lysosomal membranes to form pores causing the diffusion of protons, ions, and hydrolases that can trigger inflammation and cell death.

Damaged lysosomes are either cleared or repaired (3). In response to acute lysosomal damage, organisms use several mechanisms to protect cells from further amplification of the damage. Unlike severe lysosome damage that triggers autophagy to clear abnormal lysosomes, mild lysosomal damage initiates distinct repair mechanisms due to enhanced Ca^{2+} , ubiquitin, and lipid signals on the lysosomal surface (2). Lysosomal repair either removes the damaged site or replenishes the membrane with new lipids. To repair the damage, lipids from lysosome and ER

contact sites initiate Ca^{2+} and phosphatidylinositol 4-kinase 2a (PI4K2A) signaling. This process generates phosphatidylinositol 4-phosphate on the damaged lysosomal membrane and leads to the enrichment of several lipid transfer proteins known as OSBP-related proteins that mediate phosphatidylserine lipid transfer from the ER to damaged lysosomes (4, 5).

Damaged lysosomal membrane repair is rapid and requires bulk lipid transfer through the bridge-like autophagy protein ATG2 at the ER and lysosome contact site. However, it is unclear if ATG2 requires a lipid scramblase that flips lipids to complete membrane repair. Importantly, the related process of autophagic phagophore formation involves both ATG2 and the lipid scramblase ATG9A that together regulate phagophore expansion (6). The transmembrane protein ATG9A traffics between the trans-Golgi network and endosomes, and ATG9A-enriched vesicles transport to the phagophore that is the nascent membrane contact site after autophagy induction (7). While ATG9 has long been known to function autophagosome formation, it has been unclear if ATG9 functions in ATG2-mediated lysosomal repair. Interestingly, recent studies by Peng et al. (8) and De Tito et al. (9) both revealed that ATG9 is required for damaged lysosome repair that is independent of its role in autophagy and that this lysosomal function is conserved between *Caenorhabditis elegans* and mammals (Fig. 1).

EPG5 regulates autophagosome and lysosome fusion as a Rab7 effector across animal species (8). *epg-5* mutant *C. elegans* cells exhibit failure of autophagic substrate clearance (10) and an increase in damaged lysosomes (8). Peng et al. conducted a genetic screen for suppressors of *epg-5* mutant autophagy-defective phenotypes in *C. elegans* (8). They discovered that a mutation in the transmembrane domain 4 (TM4) of ATG-9 C475F restores autophagic substrate clearance in *epg-5* mutant worm embryos. By contrast, *atg-9* null mutants fail to restore autophagy in *epg-5* mutant worms. Interestingly, ATG-9 C475F mutant cells possessed a decrease in damaged lysosomes based on exposure of the glycan-binding protein galectin-3 in *epg-5* mutant worms, while *atg-9* null mutants failed to suppress this *epg-5* mutant phenotype. Similarly, De Tito et al. discovered that acute lysosomal damage in human cells caused by the lysosomotropic agent L-Leucyl-L-leucine methyl ester triggered PI4K2A translocation to the lysosome by ATG9A to reduce galectin-3-labeled damaged lysosomes (9). Mechanistically, this process requires the generation of phosphatidylinositol 4-phosphate by PI4K2A on damaged lysosomes that further activates the phosphoinositide-initiated membrane tethering and lipid transport pathway. At a later stage, ARFIP2 and AP-3 suppress the repair pathway to retrieve ATG9A from the lysosome.

Despite the evolutionary conservation of ATG9 function in lysosome repair, ATG9

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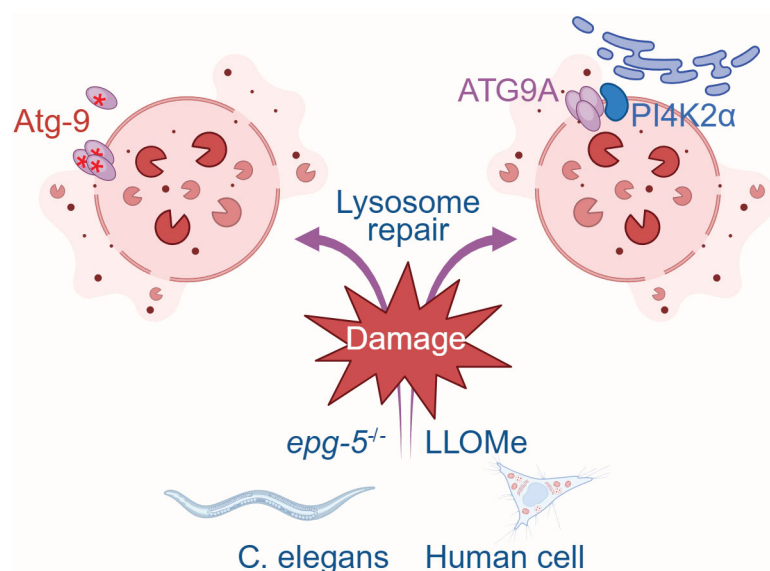


Figure 1. ATG9 evolved distinct functions in lysosome repair in *C. elegans* and human cells. Scramblase mutant Atg-9 with red asterisk restores lysosome function in *epg-5* mutant worms. By contrast, ATG9A (no asterisk) facilitates PI4K2 α recruitment to the damaged lysosome and activates phosphoinositide-initiated membrane tethering and the lipid transport pathway to repair lysosomes in human cells. LLOMe, L-Leucyl-L-leucine methyl ester.

scramblase function bifurcates in worm and human cells. The ATG9A TM4 domain is essential for lipid scrambling (6). Peng et al. revealed that C475 and conserved adjacent amino acid mutations in TM4 restore autophagy in *epg-5* mutant worm cells, thus suggesting scramblase function is not required for lysosome repair in worms. By contrast, human ATG9A scramblase mutations fail to ameliorate lysosomal damage after L-Leucyl-L-leucine methyl ester treatment (9), suggesting that ATG9 possesses potential divergent functions in lysosome repair between worms and humans.

The processes of autophagy and lysosome reformation are related (2), and lysosome reformation compensates for the loss of damaged lysosomes. Interestingly, *C. elegans* possess a mechanism by which phosphatidylethanolamine (PE) limitation restricts lysosome size and suppresses

damaged lysosomes in *epg-5* mutant worms. Since PE contributes to autophagosome formation (7), the suppression of PE production limits the extent of cargoes delivered to lysosomes by autophagy. This process may maintain materials for cell function without consuming functional lysosomes in animals.

ATG9 was discovered as a regulator of autophagy in yeast, but it is unclear if organisms that are more primitive than animals possess a lysosome damage repair mechanism. It is interesting that clear differences exist between worm and human lysosome repair mechanisms despite the common function of ATG9 in this process. Thus, many questions remain unanswered, including if organisms such as plants and fungi with their single large lysosome-equivalent vacuole possess a similar repair mechanism. It is also important to

determine how ATG-9 regulates lysosome repair independent of its scramblase function in worms and how low PE levels restrict lysosomal size to avoid lysosomal damage. Lysosome function is important in many diseases, and it is interesting to consider the possibility that ATG9A could be a therapeutic target for human lysosomal diseases.

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