New scissor for lysosome

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The lysosome is an organelle found in almost all types of eukaryotic cells. It is responsible for breaking down macromolecules, old cell components, and microorganisms. Serving as both a degradation/recycling and signaling center within cells, the lysosome plays a crucial role in regulating cell and tissue homeostasis, body development, and aging.[1] Discovered in the 1950s by Belgian scientist Christian De Duve,[2] extensive research has been conducted to understand the structure and function of lysosomes. Much of our knowledge about lysosomes comes from studying cultured cells. Progress in understanding its specific roles in processes like maintaining homeostasis, development, and aging has been slow due to the lack of effective in vivo research systems.

Lysosomes undergo constant fusion and fission processes, leading to dynamic changes in their structure and contents. These processes enable lysosomes to communicate with other membrane organelles, which are critical to maintain lysosomal luminal contents for substrate degradation, self-regeneration and secretion of substances. Each of these functions is tailored for different cell types. Lysosomes fuse with other organelles to obtain necessary components, acidity, and degrade cargoes, while they undergo fission to regulate their number, shape, size, composition, and function. The fusion of lysosomes with cargo-containing vesicles requires N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes, while the mechanism of the fission process remains largely unclear.

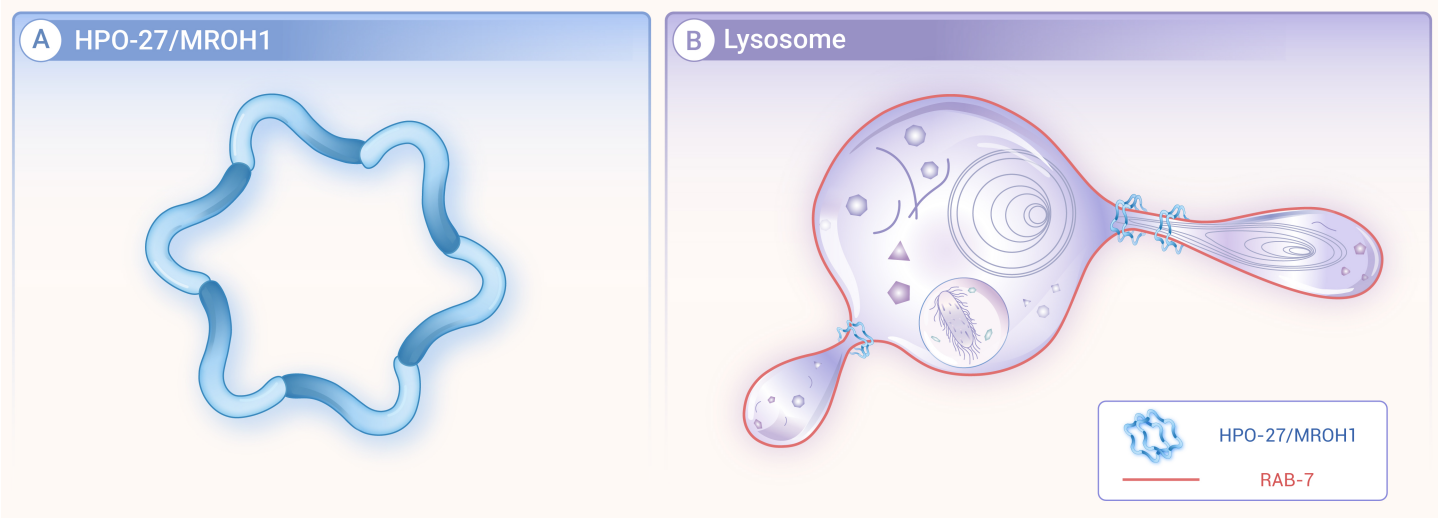

Figure 1. HPO-27/MROH1 forms helical formations around the lysosome and self-assembles in a head-to-tail mode (A) HPO-27/MROH1 self-assembles in a head-to-tail mode. (B) HPO-27/MROH1 forms helical formations around the lysosome to cut it.

Various factors have been linked to the process of lysosome fission, including components of the vesicle-forming machinery, several hereditary spastic paraplegia proteins (SPG11, SPG15, and AP5), phosphatidylinositol 3-phosphate (PtdIns3P), phosphatidylinositol 4-phosphate (PtdIns4P), phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2), phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,5)P2), dynamin superfamily proteins, and others. However, there is no direct demonstration of the specific molecules responsible for lysosome fission.

Li and colleagues have recently reported that the HEAT repeat protein HPO-27 is a key protein responsible for lysosome fission.[3] The researchers developed a system using the *C. elegans* model to investigate lysosomes in a multicellular context, focusing on their morphology and functions during development. In *C. elegans*, lysosomes displayed both vesicular and tubular shapes, with a predominance of vesicular structures and some short tubules. By conducting unbiased genetic screening, they identified a mutation in the gene encoding the HEAT repeat protein HPO-27, similar to the human MROH1 protein, which led to an increase in lysosomal tubulation. HPO-27 and MROH1 are large proteins with 37 HEAT repeats and no other identifiable domains. The researchers confirmed the lysosomal phenotype of *hpo-27* mutants using transmission electron microscopy (TEM). In wild-type cells, lysosomes appeared as dense, spherical vesicles enclosed by membranes, whereas *hpo-27* mutants showed a decrease in dense vesicular lysosomes and an increase in lysosomal tubules. The excessive tubules formed networks that ruptured to release their contents. Interestingly, the ER network was disrupted, and mitochondria were fragmented and enlarged in *hpo-27* mutants. Since HPO-27 is not localized to the ER or mitochondria, suggesting that lysosomal rupture might damage other organelles. Loss of *hpo-27* affected lysosomal activity, leading to impaired degradation of autophagic and phagocytic cargoes.

Hyper-tubulation can be a result of excessive fusion or defective fission. In order to investigate how HPO-27 influences lysosome dynamics, the researchers utilized high-resolution time-lapse imaging to observe the movement of HPO-27::mKate2. The findings indicated that HPO-27 binded to lysosomes and accumulated at specific points to facilitate the separation of tubules. Since dynamin is a key player in membrane fission, the researchers then examined whether dynamin is necessary for lysosomal fission. While a mutation in *dyn-1* led to a slight increase in the number and length of lysosomal tubules, it did not impact the *hpo-27* phenotype or the positioning of HPO-27 on lysosomes. Additionally, DYN-1 was not found to be associated with lysosomes, suggesting that dynamin does not have a significant role in lysosome fission.

Next, they asked if the function of HPO-27 is conserved. When MROH1 was introduced externally in the worm, it located to the lysosome. Furthermore, it also localized to the lysosome in mammalian cells. Knocking out

MROH1 led to more tubules and longer total length of the lysosomes, while decreasing tubule fission events. Additionally, the absence of MROH1 impacted the acidity and proteolytic function of lysosomes, indicating its importance in mammalian cells.

Lysosomal fission requires HPO-27 to be recruited to the lysosomal membrane. The question arises as to how HPO-27 is able to be present in lysosomes despite lacking a transmembrane domain. The authors showed that RAB-7, a lysosome localized small G-protein, colocalized with HPO-27 on lysosomes. Furthermore, the presence of GDP-bound inactive RAB-7(T23N) disrupted the association of lysosomes with HPO-27, while GTP-bound active RAB-7(Q68L) significantly increased this association. Similar colocalization was also observed with MROH1, suggesting that HPO-27 and MROH1 function as effectors of RAB-7 and are recruited to lysosomes through direct binding with RAB-7.

To verify HPO-27 severs membrane directly, the authors used the supported membrane tube (SMrT) assay to observe membrane fission in real time.[4] HPO-27–eGFP and MROH1-eGFP were observed to create distinct puncta along the SMrTs, and they were able to constrict and cut the membrane tubes, suggesting their direct ability to sever membranes in vitro. Through single-molecule imaging, it was suggested that HPO-27 and MROH1 might self-assemble. Negative-staining electron microscopy (EM) and 2D classification revealed that some HPO-27/MROH1 molecules took on a curved linear shape, resembling the predicted hook-like monomeric structure by RoseTTAFold,[5] suggesting the formation of higher order (oligomerization). Then, photobleaching and single-molecule imaging assay showed that HPO-27-eGFP does form oligomers. Furthermore, the authors propose that HPO-27 molecules might interact in a head-to-tail manner. This hypothesis was confirmed through tripartite split-GFP assays. The N or C-terminal HEAT repeat domain was essential for its self-assembly, as its absence prevented the formation of HPO-27 polymers and the membrane-splitting activity. Therefore, HPO-27 and MROH1 formed helical formations around the tube (Figure 1A) and self-assembled in a head-to-tail mode (Figure 1B). Disruption the head-to-tail interaction of HPO-27 by deleting the N or C-terminal HEAT repeat domain blocked its association with lysosomes, suggesting the self-assembly process plays a crucial role in the lysosomal connection of HPO-27 and could enhance its interactions with active RAB-7.

The importance of HPO-27 from a physiological perspective lies in its phenotype of dead embryos and larval arrest in its mutants. Additionally, it is essential for cuticle replacement during molting and the normal lifespan of the worm. Furthermore, HPO-27 plays a crucial role in clearing protein aggregates by facilitating lysosome fission.

In summary, the authors have identified HPO-27 as a molecule responsible for lysosome scission, and found that HPO-27 and MROH1 self-assembly to facilitate membrane constriction and scission. Interestingly, they were able to separate SMrTs without requiring direct energy input, which sets them apart from the traditional membrane scissor dynamin. This discovery introduces a new group of scission factors and opens up avenues for further research on lysosome fission linked to HPO-27. Future investigations into HPO-27 are expected to shed light on mechanisms governing lysosomal membrane balance and offer fresh insights for treating associated diseases. Numerous unanswered questions remain, such as whether HPO-27 and MROH1 form a head-to-tail structure in living organisms, how they self-assemble, whether any molecules assist in their assembly *in vivo*, whether and how HPO-27/MROH1 cooperate with partners to regulate lysosome fission, and if they consume energy directly in vivo, whether PA, PtdIns3P, PtdIns4P and PtdIns(4,5)P2 regulate HPO-27 targeting and assembly at scission sites.

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DECLARATION OF INTERESTS

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