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# Rab GTPases mature the LC3-associated midbody phagosome

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**ABSTRACT**

Post-mitotic midbody remnants have recently been added to the list of structures degraded via LC3-associated phagocytosis (LAP). LAP involves proteins of the autophagy pathway to degrade phagosomal contents, mingling 2 pathways that were thought to be distinct. To better characterize how similar LAP is to classical phagocytosis, we asked whether the midbody LAPosome (LC3-associated phagosome) matures using Rab GTPases in *C. elegans* embryos. We found that RAB-5 and RAB-7 appear transiently on midbody LAPosomes and that RAB-7 is required for midbody degradation, suggesting that RAB-5 and RAB-7 direct LAPosome maturation similar to classical phagosomes. Further, we observed that the Rab2 homolog UNC-108 and the major proton pump, the V-type ATPase, are required for acidification of the midbody LAPosome, demonstrating that phagosomes and LAPosomes acidify via a common pathway. Together, these data reveal that Rab GTPases play similar roles during LAPosome maturation and phagosome maturation.

## Introduction

Phagocytosis and autophagy degrade cargos of different origins. Cells use phagocytosis to ingest extracellular particles, extending the plasma membrane to isolate particles in a phagosome membrane. Macroautophagy (hereafter referred to as autophagy) is used to wrap cytosolic particles in a double membrane, isolating them from the cytoplasm. Given the different origin of the degraded cargo, it was thought that phagocytosis and autophagy use different molecular mechanisms.[1] However, both pathways converge on the lysosome and certain proteins involved in autophagy have also been shown to be required for phagosome degradation. This led to the proposal of LC3-associated phagocytosis (LAP), where autophagy-associated proteins including the ubiquitin-like Atg8/LC3 family appear on phagosomes and facilitate their fusion with lysosomes.[2]

Recently, we discovered that post-mitotic midbody remnants are degraded by LAP.[3] The midbody is formed in the cytosol during cell division, but released extracellularly by abscission as the midbody remnant (referred to hereafter as midbody).[4-6] The post-mitotic regulation of midbodies is of particular interest because their positioning can affect cell polarity and cell fate.[7-10] Previously, it was thought that cytosolic midbodies were degraded by autophagy and that released midbodies were phagocytosed and degraded by an Atg8/LC3-

independent pathway.[7,11-12] To test these hypotheses, we systematically examined 5 different models for midbody degradation in *C. elegans* embryos (Fig. 1) and demonstrated that only LAP was consistent with all the data in the field.[3]

The classical phagocytosis model proposes that released midbodies are internalized by phagocytosis. The phagosome then matures using early and late endosomal GTPases Rab5 and Rab7 before fusing with a lysosome for degradation[13] (Fig. 1A). Although this model was supported by the evidence for release and phagocytosis of the midbody,[3-5] it does not explain the appearance of autophagy-related proteins on the midbody nor the role of autophagy-associated proteins in midbody degradation.[3,7,11] The macroautophagy model suggests that abscission occurs on one side of the midbody before it is released into the cytoplasm. A phagophore membrane then isolates the midbody, whose elongation is mediated by the class III PI3K. Fusion of autophagosomes with lysosomes depend on the Atg8/LC3 family proteins LGG-1/2 and the RAB-7 GTPase[14,15] (Fig. 1B). However, 3 pieces of evidence speak against the macroautophagy model. First, using a degradation tag on a midbody ring component, we revealed that the midbody is completely released after symmetric abscission and is not exposed to the cytoplasm in wild type embryos or autophagy mutants.[3] Second, others and we have shown that the
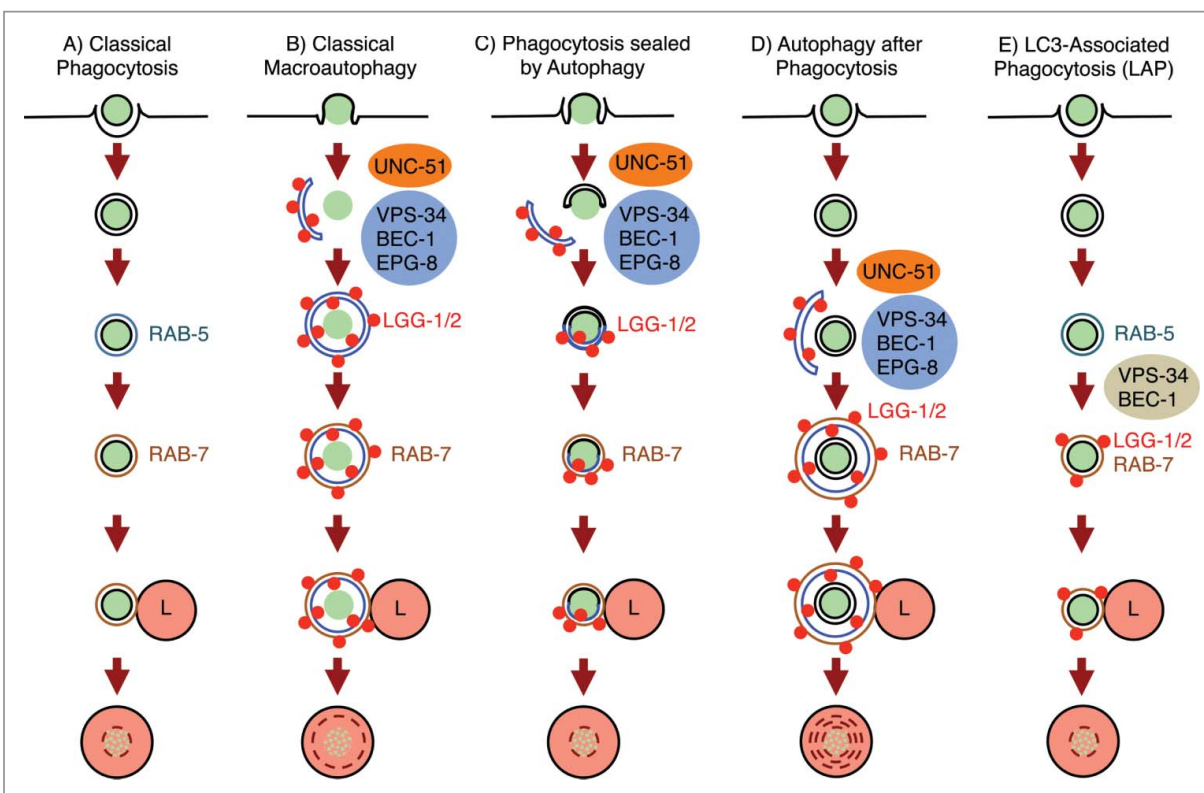
**Figure 1.** Models for midbody internalization and degradation. (A) Under the classical phagocytosis model, released midbodies are phagocytosed, and the phagosome matures using RAB-5 (light blue) and RAB-7 (brown) sequentially before fusing with a lysosome (L) for degradation. (B) The macroautophagy model suggests that abscission occurs on one side of the midbody before it is released into the cytoplasm. The pre-initiation factor UNC-51/ULK1 and the class III PI3K complex are required for formation of the LGG-2/LC3-containing phagophore membrane (dark blue) that wraps the midbody. The autophagosome recruits RAB-7 (brown), which mediates tethering and fusion with a lysosome together with the Atg8/LC3 homologs LGG-1 and LGG-2 (red). (C) In the phagocytosis sealed by autophagy model, phagocytosis internalizes the midbody after asymmetric abscission. A phagophore seals the exposed midbody. (D) In the autophagy after phagocytosis model, the midbody phagosome is wrapped by a phagophore, resulting in a quadruple membrane structure. (E) In the LC3-associated phagocytosis (LAP) model, the released midbody is phagocytosed; the phagosome matures by RAB-5 and RAB-7 and acquires Atg8/LC3 family proteins LGG-1/2 by a PI3K complex that lacks the Atg14 homolog EPG-8. The LC3-associated phagosome (LAPosome) uses both RAB-7 and LGG-1/2 to fuse with a lysosome and degrade the midbody.

midbody is internalized via receptor-mediated phagocytosis.[3,4] Third, we have shown that the autophagy pre-initation factor UNC-51/ULK1 and the macroautophagy-specific PI3K subunit EPG-8/Atg14[16] are dispensable during midbody degradation.[3] Thus, neither the phagocytosis nor the macroautophagy model was supported by all the data.

We then considered 3 alternative hypotheses, which fused aspects of phagocytosis and autophagy (Fig. 1C–E). In the "phagocytosis sealed by autophagy" model, the midbody is phagocytosed after asymmetric abscission. Then, a phagophore membrane seals the part of the midbody exposed to the cytoplasm (Fig. 1C). However, this model would also require autophagy-specific proteins, such as UNC-51 and EPG-8, which were not needed for midbody degradation.[3] Furthermore, using a degradation tag, we revealed that the midbody was not exposed to the cytoplasm, even in autophagy mutants like *atg-7*

knockdowns.[3] In the "autophagy after phagocytosis" model, the midbody phagosome is further wrapped by a phagophore, resulting in a quadruple membrane structure (Fig. 1D). Although this model would be consistent with the degradation tag data as the midbody would not be exposed to the cytoplasm, it is ruled out by the observation that midbody degradation is not delayed in *unc-51* and *epg-8* mutants.[3] We finally considered whether a non-canonical form of autophagy, LC3-associated phagocytosis (LAP), would be consistent with the observed data. The LAP model of midbody degradation suggests that after symmetric abscission, the released midbody is internalized by phagocytosis (Fig. 1E). The phagosome acquires LGG-1/2 by a PI3K complex that lacks the Atg14 homolog EPG-8. The LC3-associated phagosome (LAPosome) later fuses with a lysosome to degrade the midbody. This model is consistent with the observed roles of phagocytic and autophagy-related proteins during midbody

acidification, starting with the Rab2 homolog UNC-108. We used a GFP(S65C)-tagged midbody reporter, which loses fluorescence at lower pH.[3] While NMY-2::GFP(S65C)::ZF1 disappeared from P0 midbodies 22 ± 7 min after internalization in control embryos (n = 17), P0 midbodies in *unc-108* RNAi-treated embryos lost fluorescence 34 ± 13 min after internalization (n = 9, Fig. 2D). As LGG-2/LC3 rapidly appears on midbody phagosomes after internalization,[3] the observed delay in acidification demonstrates that the Rab2 homolog UNC-108 acidifies both phagosomes and LAPosomes.

It was proposed that UNC-108 could regulate acidification of phagosomes by recruiting the main proton pump in the cell, the V-type ATPase.[20] Therefore, we tested whether acidification of midbody LAPosomes depends on the V-type ATPase. We targeted VHA-1, an essential subunit of the $V_0$ proton translocation domain.[21] Long-term treatment with *vha-1* RNAi results in larval arrest or sterility,[22] so we performed "germ line-specific" RNAi treatments using the *rrf-1* mutant,[23] similar to previous experiments with *rab-5* RNAi.[3] Disappearance of the NMY-2::GFP(S65C)::ZF1 reporter was delayed after treating worms with *vha-1* RNAi (39 ± 13 min, n = 9, Fig. 2D). This delay was not due to the *rrf-1* mutant background, because acidification of the P0 midbody was normal in untreated *rrf-1* mutants (22 ± 8 min, n = 9). Thus, these data demonstrate that the V-type ATPase is required for midbody LAPosome acidification.

## Discussion

Our data reveal the roles of Rab GTPases during internalization and maturation of the midbody LAPosome (Fig. 3). We predict that RAB-5 acts at 2 separate stages during LAP. First, RAB-5 is required for the trafficking of the phagocytic receptor CED-1 to the plasma membrane and thereby midbody phagocytosis.[3] Second, after internalization, RAB-5 appears on the midbody LAPosome and is rapidly exchanged for RAB-7. Thus, although the process occurs more quickly, RAB-5 is likely to recruit RAB-7 to mature the midbody LAPosome similar to phagosome maturation during cell corpse clearance in *C. elegans*.[18-20] Studying midbody degradation is therefore likely to yield insights into the mechanisms of cell corpse clearance during development and homeostasis.

RAB-7 is required for lysosome tethering and localizes on the midbody LAPosome for a considerably longer time than RAB-5 (Fig. 3). During the last steps of autophagy, autophagosomes acquire PLEKHM1, an adaptor protein with binding sites for both RAB-7 and LC3.[14] PLEKHM1 subsequently recruits the HOPS complex (homotypic fusion and vacuole protein sorting), which is required to tether autophagosomes with lysosomes in worms and mammals.[14,24] As RAB-7 and the Atg8/LC3 homologs are recruited to the midbody phagosome and required for midbody degradation (Fig. 2C and ref. 3), we speculate that RAB-7 and the Atg8/LC3 homologs will recruit the PLEKHM1 homolog and the HOPS complex to tether midbody LAPosomes with lysosomes for degradation.

Our data demonstrate that the Rab2 homolog UNC-108 is required for the timely acidification of the midbody LAPosome (Fig. 3). UNC-108 is likely to act in parallel with other Rab GTPases to traffic proteins to or from the LAPosome for acidification. For example, UNC-108 and RAB-14 are both recruited to cell corpse phagosomes and are redundantly required to regulate
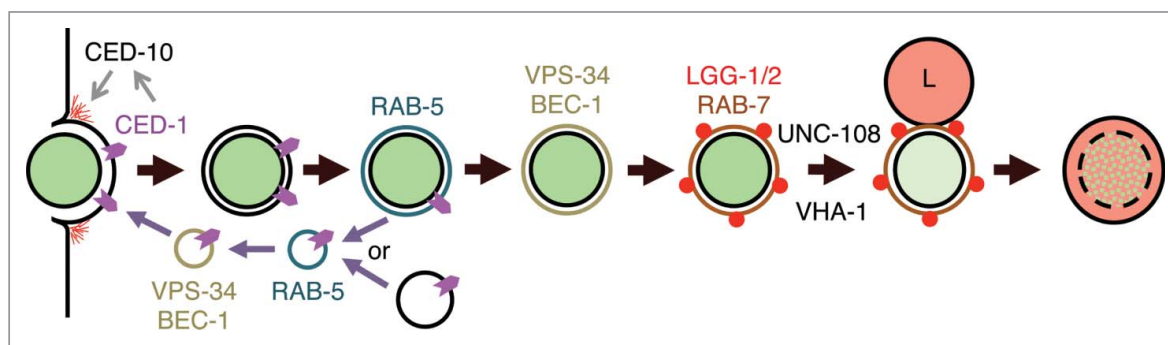


**Figure 3.** Rab GTPases act at different steps during midbody internalization and degradation. Midbody phagocytosis depends on the plasma membrane receptor CED-1/MEGF11, which induces actin polymerization and formation of a phagocytic cup by CED-10/Rac1. RAB-5 and its effector PI3K complex (including VPS-34 and BEC-1/Beclin1) regulate recycling of CED-1 from multiple sources to the plasma membrane. After internalization, the phagosome membrane acquires RAB-5 and then RAB-7. RAB-5 also attracts PI3K, which later recruits the Atg8/LC3 family proteins LGG-1/2. The LAPosome contents acidify in an UNC-108/Rab2- and V-ATPase-dependent manner before fusion with a lysosome (L) and degradation.

the acidification of cell corpse phagosomes.[20] One candidate cargo of UNC-108 and RAB-14 trafficking is the endosomal V-type ATPase, a large transmembrane proton pump known as the main acidifier in cells.[25] Our data showing that acidification is delayed in *unc-108* and *vha-1* mutants are consistent with the hypothesis that UNC-108 could mediate acidification of midbody LAPosomes through trafficking the V-type ATPase. However, LAPosome acidification is independent of Atg8/LC3, because the midbody phagosome acidified normally in *atg-7* mutants.[3] Thus, UNC-108 and the V-type ATPase are able to acidify LAPosomes irrespective of Atg8/LC3 on the phagosome surface.

Since the first description of LAP, it is becoming clear that proteins used as hallmarks of autophagy like Atg8/LC3 can also be recruited to phagosomes to facilitate their fusion with lysosomes. Although, some phagosomes can tether with lysosomes independent of LC3,[26] LC3 binding to adaptors such as PLEKHM1 may aid tethering. Further investigation is needed to understand why LAPosomes require LC3 to tether with lysosomes. As multiple membranes usually wrap LAPosomes (often 2 plasma membrane-derived vesicles), we speculate that LC3 is needed to signal to lysosomes that the LAPosome contains internal membranes that require degradation or fusion. LC3 could also recruit a subtype of lysosomes that are specialized for the degradation of multi-membrane wrapped cargo. LC3 would then play a similar role in the degradation of double-membrane wrapped autophagosomes.

## Materials and methods

### Worm strains and maintenance

*Caenorhabditis elegans* strains were maintained according to standard protocols.[27] The following strains were used in this study: FT23: *xnIs8 [pJN343: nmy-2p::NMY-2::mCherry; unc-119(+)] unc-119(ed3) III*,[28] RT122: *unc-119(ed3) III; pwIs20 [pie-1p::gfp::rab-5; unc-119(+)]*,[29] RT123: *unc-119(ed3) III; pwIs21 [pie-1p::gfp::rab-7; unc-119(+)]*,[30] WEH02: *ltIs38 [pie-1p::GFP::PH(PLC1∂1); unc-119(+)] xnIs8 [pJN343: nmy-2p::NMY-2::mCherry; unc-119(+)] unc-119(ed3) III*,[3] WEH51: *unc-119(ed3) III; xnIs65 [nmy-2p::nmy-2::gfp::zf1; unc-119(+)] IV; ltIs44 [pie-1p::mCherry::PH(PLC1∂1); unc-119(+)] V*,[3] WEH141: *rrf-1(pk1417) I; unc-119(ed3) III; xnIs65 [nmy-2p::nmy-2::gfp::zf1; unc-119(+)] IV; ltIs44 [pie-1p-mCherry::PH(PLC1∂1); unc-119(+)] V*,[3] WEH212: *xnIs8 [pJN343: nmy-2p::NMY-2::mCherry; unc-119(+)] unc-119(ed3) III; pwIs20 [pie-1p::gfp::rab-5; unc-119(+)]*, and WEH214: *xnIs8 [pJN343: nmy-2p::NMY-2::mCherry; unc-119(+)] unc-119(ed3) III; pwIs21 [pie-1p::gfp::rab-7, unc-119(+)]*. WEH212 and WEH214 were generated by crossing FT23 with RT122 and RT123, respectively.

### RNAi experiments

*unc-108* and *vha-1* RNAi were performed by feeding worms dsRNA-expressing bacteria from the L1 larval stage through adulthood at 25°C (60–70 h) according to established protocols.[31] For *rab-7* RNAi, L3/L4 worms were treated at 25°C for 18–25 h as longer treatment caused sterility. Control animals were fed OP50. RNAi bacteria were obtained from available libraries (Source BioScience). The following clones were used: *rab-7* (mv_W03C9.3), *unc-108* (mv_F53F10.4), and *vha-1* (mv_R10E11.8).

### Time-lapse imaging

Embryos were dissected from gravid adults and mounted in M9 buffer on an agarose pad on a slide. For the colocalization analysis in Fig. 2A–B, live embryos were imaged simultaneously using a Leica TCS SP5 confocal microscope with a HCX PL APO 63× 1.4 NA oil objective lens supplemented with a Leica HyD hybrid detector. Z-stacks were collected every 0.5 $\mu$m every min. For RAB-5 and RAB-7 timing and the acidification/degradation analysis in Fig. 2C–D, Z-stacks were acquired sequentially for GFP and then mCherry every 1.2 $\mu$m every 20 seconds using a Leica DM5500 wide-field fluorescence microscope with a HC PL APO 40× 1.3 NA oil objective lens supplemented with a Leica DFC365 FX CCD camera controlled by LAS AF software.

### Image analysis and manipulation

Time-lapse series were analyzed using Imaris (Bitplane). Internalization is defined as the first frame where the midbody moves away from the plasma membrane. For clarity, images were rotated, colorized to cyan and yellow, and the intensity was adjusted using Adobe Photoshop. In Fig. 2A–B, 4 Z-slices were projected (Z interval of 0.5 $\mu$m), except only one Z-plane is shown in inset images.

### Statistical evaluation

Student's one-tailed *t*-test was used to test statistical significance. Mean ± standard error of the mean is depicted in Fig. 2C–D. Mean ± standard deviation is reported in the text.

## Disclosure of potential conflicts of interest

## Author contributions

GF performed all experiments. GF and AMW designed and analyzed all experiments and wrote the manuscript.

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## References

[1] Oczypok EA, Oury TD, Chu CT. It's a Cell-Eat-Cell World Autophagy and Phagocytosis. Am J Pathol 2013; 182:612-622; PMID:23369575; http://dx.doi.org/10.1016/j.ajpath.2012.12.017

[2] Mehta P, Henault J, Kolbeck R, Sanjuan MA. Noncanonical autophagy: one small step for LC3, one giant leap for immunity. Curr Opin Immunol 2014; 26:69-75; PMID:24556403; http://dx.doi.org/10.1016/j.coi.2013.10.012

[3] Fazeli G, Trinkwalder M, Irmisch L, Wehman AM. C-elegans midbodies are released, phagocytosed and undergo LC3-dependent degradation independent of macroautophagy. Journal of Cell Science 2016;129:3721-3731; PMID:27562069; http://dx.doi.org/10.1242/jcs.190223

[4] Ou G, Gentili C, Gonczy P. Stereotyped distribution of midbody remnants in early C. elegans embryos requires cell death genes and is dispensable for development. Cell Res 2014; 24:251-253.

[5] Crowell EF, Gaffuri AL, Gayraud-Morel B, Tajbakhsh S, Echard A. Engulfment of the midbody remnant after cytokinesis in mammalian cells. J Cell Sci 2014; 127:3840-3851; PMID:25002399; http://dx.doi.org/10.1242/jcs.154732

[6] Arai Y, Sampaio JL, Wilsch-Brauninger M, Ettinger AW, Haffner C, Huttner WB. Lipidome of midbody released from neural stem and progenitor cells during mammalian cortical neurogenesis. Front Cell Neurosci 2015; 9:325

[7] Kuo TC, Chen CT, Baron D, Onder TT, Loewer S, Almeida S, Weismann CM, Xu P, Houghton JM, Gao FB, Daley GQ, Doxsey S. Midbody accumulation through evasion of autophagy contributes to cellular reprogramming and tumorigenicity. Nat Cell Biol 2011; 13:1214-1223; PMID:21909099; http://dx.doi.org/10.1038/ncb2332

[8] Morais-de-Sa E, Sunkel CE. Connecting polarized cytokinesis to epithelial architecture. Cell Cycle 2013; 12:3583-3584; PMID:24189532; http://dx.doi.org/10.4161/cc.26910

[9] Singh D, Pohl C. Coupling of rotational cortical flow, asymmetric midbody positioning, and spindle rotation mediates dorsoventral axis formation in C. elegans. Dev Cell 2014; 28:253-267

[10] Bernabe-Rubio M, Andres G, Casares-Arias J, Fernandez-Barrera J, Rangel L, Reglero-Real N, Gershlick DC, Fernandez JJ, Millan J, Correas I, Miguez DG, Alonso MA. Novel role for the midbody in primary ciliogenesis by polarized epithelial cells. J Cell Biol 2016; 214:259-273; PMID:27458130; http://dx.doi.org/10.1083/jcb.201601020

[11] Pohl C, Jentsch S. Midbody ring disposal by autophagy is a post-abscission event of cytokinesis. Nat Cell Biol 2009; 11:65-70; PMID:19079246; http://dx.doi.org/10.1038/ncb1813

[12] Isakson P, Lystad AH, Breen K, Koster G, Stenmark H, Simonsen A. TRAF6 mediates ubiquitination of KIF23/MKLP1 and is required for midbody ring degradation by selective autophagy. Autophagy 2013; 9:1955-1964; PMID:24128730; http://dx.doi.org/10.4161/auto.26085

[13] Fairn GD, Grinstein S. How nascent phagosomes mature to become phagolysosomes. Trends Immunol 2012; 33:397-405; PMID:22560866; http://dx.doi.org/10.1016/j.it.2012.03.003

[14] McEwan DG, Popovic D, Gubas A, Terawaki S, Suzuki H, Stadel D, Coxon FP, de Stegmann DM, Bhogaraju S, Maddi K, Kirchof A, Gatti E, Helfrich MH, Wakatsuki S, Behrends C, Pierre P, Dikic I. PLEKHM1 Regulates Autophagosome-Lysosome Fusion through HOPS Complex and LC3/GABARAP Proteins. Molecular Cell 2015; 57:39-54; PMID:25498145; http://dx.doi.org/10.1016/j.molcel.2014.11.006

[15] Djeddi A, Al Rawi S, Deuve JL, Perrois C, Liu YY, Russeau M, Sachse M, Galy V. Sperm-inherited organelle clearance in C-elegans relies on LC3-dependent autophagosome targeting to the pericentrosomal area. Development 2015; 142:1705-1716; PMID:25922527; http://dx.doi.org/10.1242/dev.117879

[16] Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletier S, Gingras S, Orchard R, Guan JL, Tan H, Peng J, Kanneganti TD, Virgin HW, Green DR. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat Cell Biol 2015; 17:893-906; PMID:26098576; http://dx.doi.org/10.1038/ncb3192

[17] Salzmann V, Chen C, Chiang CY, Tiyaboonchai A, Mayer M, Yamashita YM. Centrosome-dependent asymmetric inheritance of the midbody ring in Drosophila germline stem cell division. Mol Biol Cell 2014; 25:267-275; PMID:24227883; http://dx.doi.org/10.1091/mbc.E13-09-0541

[18] Florey O, Kim SE, Sandoval CP, Haynes CM, Overholtzer M. Autophagy machinery mediates macroendocytic processing and entotic cell death by targeting single membranes. Nat Cell Biol 2011; 13:1335-1343; PMID:22002674; http://dx.doi.org/10.1038/ncb2363

[19] Kinchen JM, Doukoumetzidis K, Almendinger J, Stergiou L, Tosello-Trampont A, Sifri CD, Hengartner MO, Ravichandran KS. A pathway for phagosome maturation during engulfment of apoptotic cells. Nat Cell Biol 2008; 10:556-566; PMID:18425118; http://dx.doi.org/10.1038/ncb1718

[20] Guo P, Hu T, Zhang J, Jiang S, Wang X. Sequential action of Caenorhabditis elegans Rab GTPases regulates phagolysosome formation during apoptotic cell degradation. Proc Natl Acad Sci U S A 2010; 107:18016-18021; PMID:20921409; http://dx.doi.org/10.1073/pnas.1008946107

[21] Lee SK, Li W, Ryu SE, Rhim T, Ahnn J. Vacuolar (H+)-ATPases in Caenorhabditis elegans: What can we learn about giant H+ pumps from tiny worms? Bba-Bioenergetics 2010; 1797:1687-1695; PMID:20637717; http://dx.doi.org/10.1016/j.bbabio.2010.07.004

[22] Simmer F, Moorman C, van der Linden AM, Kuijk E, van den Berghe PVE, Kamath RS, Fraser AG, Ahringer J, Plasterk RHA. Genome-wide RNAi of C-elegans using the hypersensitive rrf-3 strain reveals novel gene functions. Plos Biology 2003; 1:77-84; http://dx.doi.org/10.1371/journal.pbio.0000012

[23] Sijen T, Fleenor J, Simmer F, Thijssen KL, Parrish S, Timmons L, Plasterk RH, Fire A. On the role of RNA amplification in dsRNA-triggered gene silencing. Cell 2001; 107:465-476; PMID:11719187; http://dx.doi.org/10.1016/S0092-8674(01)00576-1

[24] Jenzer C, Manil-Segalen M, Lefebvre C, Largeau C, Glatigny A, Legouis R. Human GABARAP can restore autophagosome biogenesis in a C. elegans lgg-1 mutant. Autophagy 2014; 10:1868-1872; PMID:25126728; http://dx.doi.org/10.4161/auto.29745

[25] Huynh KK, Grinstein S. Regulation of vacuolar pH and its modulation by some microbial species. Microbiol Mol Biol R 2007; 71:452-62; http://dx.doi.org/10.1128/MMBR.00003-07

[26] Cemma M, Grinstein S, Brumell JH. Autophagy proteins are not universally required for phagosome maturation. Autophagy 2016; 12:1440-1446; PMID:27310610; http://dx.doi.org/10.1080/15548627.2016.1191724

[27] Brenner S. The genetics of Caenorhabditis elegans. Genetics 1974; 77:71-94; PMID:4366476

[28] Nelson MD, Zhou E, Kiontke K, Fradin H, Maldonado G, Martin D, Shah K, Fitch DH. A bow-tie genetic architecture for morphogenesis suggested by a genome-wide RNAi screen in Caenorhabditis elegans. PLoS Genet 2011; 7:e1002010; PMID:21408209; http://dx.doi.org/10.1371/journal.pgen.1002010

[29] Sato M, Sato K, Fonarev P, Huang CJ, Liou W, Grant BD. Caenorhabditis elegans RME-6 is a novel regulator of RAB-5 at the clathrin-coated pit. Nature Cell Biology 2005; 7:559-U557; PMID:15895077; http://dx.doi.org/10.1038/ncb1261

[30] Kang J, Bai ZY, Zegarek MH, Grant BD, Lee J. Essential roles of snap-29 in C. elegans. Developmental Biology 2011; 355:77-88; PMID:21545795; http://dx.doi.org/10.1016/j.ydbio.2011.04.013

[31] Fraser AG, Kamath RS, Zipperlen P, Martinez-Campos M, Sohrmann M, Ahringer J. Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. Nature 2000; 408:325-330