GOP-1: Helping phagosomes pass the acid test

Lynda M. Stuart[1,2] and Adam Lacy-Hulbert[1]

[1]Immunology Program, Benaroya Research Institute and [2]Discovery and Translational Sciences, Bill and Melinda Gates Foundation, Seattle, WA

Phagosomes form during engulfment of large particles and become increasingly acidic and proteolytic, ultimately fusing with lysosomes, in a process termed "phagosome maturation." In this issue, Yin et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201610001) identify GOP-1 as essential for the maturation of phagosomes containing apoptotic cells, through recruitment of the Rab GTPase UNC108.

Phagocytosis is a fundamental cellular process in which particles are recognized and engulfed whole by cells. The evolutionary origin of phagocytosis is likely to be as a means of obtaining nutrients, but it now serves many diverse processes, including clearance of apoptotic cells during embryogenesis and tissue homeostasis and host defense against infection.

Phagocytosis is initiated by cell surface receptors, which trigger cytoskeletal rearrangements and extension of membrane processes that surround and engulf the particle. This leads to formation of a specialized organelle, the phagosome, generated de novo from internalized and intracellular membranes. The main function of the phagosome is to transport internalized material to fuse with degradative lysosomes. However, this simple description greatly understates the complexity of this critical organelle and its pathway through the cell. Transport to the lysosome involves multiple membrane fusion and fission events that gradually alter the composition of the phagosome membrane and luminal contents, a process known as "phagosome maturation." This allows recycling of receptors and other membrane proteins to the cell surface, where they can engage new particles, but perhaps more importantly delivers new proteins to the phagosome enabling the organelle to perform novel functions as it transitions through the cell. For example, early in their life, phagosomes are specialized for interrogation of the internalized cargo, acting as platforms for innate immune signaling. This occurs through delivery of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) to early phagosomes, which is coordinated with the activation of the phagosomal enzymes that process cargo to release PRR ligands. Additional proteases and major histocompatibility class II proteins are recruited to later phagosomes to process phagocytosed proteins for antigen presentation. Phagosomes also acquire proteins that dramatically alter the luminal environment, such as the NADPH oxidase complex, which generates reactive oxidase species to neutralize microbes, and vacuolar (V-type) ATPases, which acidify the phagosome. Acidification is a key feature of phagosome maturation and is essential for activation of many of the proteases that degrade and process internalized material. A small drop in phagosomal pH occurs in early phagosomes, but the bulk of acidification is driven by the V-ATPase and occurs in late phagosomes.

The exact composition of each phagosome and the process of maturation are determined in large part by two factors. First, dedicated phagocytes generate phagosomes that are specialized for key functions. Neutrophil phagosomes rapidly develop high levels of reactive oxygen species to drive microbicidal activity, whereas professional antigen presenting cells such as dendritic cells delay phagosome maturation and acidification to allow controlled generation and transport of peptides for loading onto major histocompatibility class proteins. Second, the internalized cargo have a major influence on phagosome function, through signals generated by the receptors that trigger phagocytosis and from PRR signals originating from the phagosome itself, which accelerate maturation in response to microbes. Some pathogens have evolved mechanisms to combat and subvert this process to their benefit, for example, hijacking phagosome maturation to generate intracellular vacuoles where they can survive and replicate for extended periods. Phagosomes are therefore highly dynamic organelles, each tailored for their individual cargo and situation.

Much of our understanding of the cell biology of phagocytosis has come from studies of the phagocytosis of cells undergoing programmed cell death. This process is highly efficient, in some cases occurring before targets have completed the cell death program, and it is estimated that many billions of cells may be removed through this process daily in our bodies. In *Caenorhabditis elegans*, the numbers are understandably smaller: 113 out of 628 somatic cells die during embryogenesis and a further 18 die during the larval stage, with additional cell death occurring in the gonad. However, this developmental cell death occurs in specific cells at defined stages and is accompanied by rapid engulfment, which follows a similar invariant pattern, allowing genetic screens to identify the pathways involved. Apoptotic cells are internalized through two pathways, both of which are triggered by binding to phosphatidylserine on the apoptotic cell. One is mediated by a scavenger receptor, CED-1 (homologous to human MEGF10), which binds the adaptor CED-6 (GULP), the other involves a phosphatidylserine receptor and integrins, which act through CED2/5/12. The Rac GTPase CED-10 and DYN-1 (dynamin) then mediate internalization and phagosome formation.

Similar screens have been used to identify mutations that prevent phagosome acidification and cause persistence of cell "corpses" within phagocytes, allowing the study of

Correspondence to Lynda M. Stuart: lstuart@benaroyaresearch.org

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 216 No. 6 1517–1519
https://doi.org/10.1083/jcb.201704047

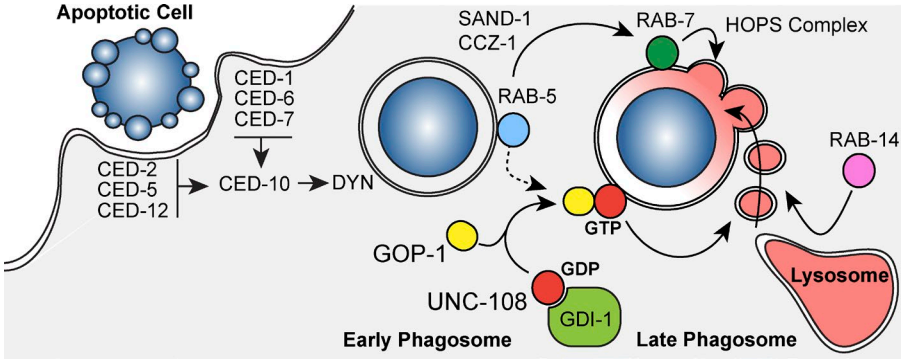

Figure 1. **Model of GOP-1/UNC-108–mediated phagosome maturation.** Apoptotic cells are phagocytosed and delivered to early phagosomes, which recruit RAB-5. GOP-1 acts as a GEF to release GDP-bound UNC-108 from GDI-1, promotes exchange of GDP for GTP, and delivers UNC-108 to the phagosome. This requires RAB-5 through an unknown mechanism (dotted line). UNC-108 then promotes recruitment of lysosomes to the phagosome, in parallel with RAB-14. RAB-5 also promotes RAB-7 recruitment to phagosomes through SAND-1/CCZ-1, which triggers lysosomal fusion with the late phagosome.

phagosome maturation in vivo. Rab proteins are a large family of small GTPases that regulate vesicular traffic in many cellular processes and four Rab proteins (RAB-5, UNC-108/Rab2, RAB-14, and RAB-7) contribute to phagosome maturation and apoptotic cell degradation. RAB-5 is recruited to early phagosomes and promotes generation of PtdIns3P through its effector VPS-34. Another RAB-5 effector, SAND-1, pairs with CCZ-1 to recruit RAB-7 to maturing phagosomes, while RAB-5 is removed. RAB-7 then promotes later phagosome maturation, including lysosomal fusion, through a process thought to involve the HOPS complex. UNC-108 and RAB-14 are also recruited to phagosomes at around the same time as RAB-7, and also in a RAB-5–dependent manner. A major function of these two Rabs is to recruit lysosomes to the phagosome, allowing RAB-7 to trigger fusion. However, although these findings have placed UNC-108 and RAB-14 into the sequence of phagosome maturation, very little is known about the regulators and effectors of these Rabs.

In this issue, Yin et al. provide an answer to the important question of what mediates the recruitment of UNC108/Rab2 to the phagosome. They identify mutations in the gene *gop-1* that cause persistence of cell corpses inside phagocytes. Mutation of *gop*-1 causes arrest of phagosome maturation at the RAB-5 to RAB-7 transition stage, prolonging RAB-5 and PtdInsP2 association with the phagosome and reducing RAB-7 recruitment. As a result, phagosomes failed to efficiently acidify, fuse with lysosomes and shrink. The phenotypes of *gop-1* mutants most closely resemble those of *unc-108* mutants, and analysis of double mutants shows these genes lie in the same pathway and work in parallel to RAB-7 and RAB-14. GOP-1 colocalizes with UNC-108 in the cell and is required for UNC-108 recruitment to the phagosome (Fig. 1).

In common with other small GTPases, Rabs switch between active, GTP-bound and inactive, GDP-bound forms. Rabs are activated during delivery to target membranes by the action of guanine nucleotide exchange factors (GEFs), which replace GDP with GTP, allowing interaction with effector proteins. GTPase-activating proteins later promote GTP hydrolysis, inactivating the Rab, which is then removed from membranes by GDP dissociation inhibitors (GDIs), to complete the cycle. Yin et al. (2017) provide considerable evidence that GOP-1 acts as a GEF for UNC-108. They show that GOP-1 binds UNC-108 in its GDP or nucleotide-free state, but not when bound to GTP. In cell-free assays, GOP-1 disrupts UNC-108 binding to its GDP-dissociation inhibitor GDI-1, promotes exchange of GDP for GTP, and mediates interaction of UNC-108 with PtdIns3P-positive membranes. Moreover, an UNC-108 mutation that facilitates GDP exchange for GTP bypasses the need for GOP-1 for phagosome recruitment and partially reverses *gop-1* mutant phenotypes. During phagocytosis, GOP-1 mediates UNC-108 activation and movement to the phagosomal membrane, where it promotes lysosome recruitment (Fig. 1). Our understanding of the mechanisms of UNC-108/Rab2 activation and action has lagged behind those for other Rabs, particularly Rab5 and Rab7, and these findings provide a key new element in this pathway. These insights also apply to other facets of membrane trafficking. UNC-108/Rab2, like RAB-5 and RAB-7, also participates in endosomal trafficking. Yin et al. (2017) show that GOP-1 is also required for UNC-108 recruitment to endosomes and *gop-1* mutants have defects in endosome–lysosome fusion and the endosomal trafficking required to form dense core vesicles in neurons. Hence, the role of GOP-1 in UNC-108 activation is not limited to phagocytosis.

Several questions remain to be answered. Foremost among these is how GOP-1 is activated and recruited to the phagosome. Yin et al. (2017) show that this requires RAB-5, but occurs through a different mechanism than the SAND1-mediated recruitment of RAB-7, suggesting involvement of a different RAB-5 effector or a more complex mechanism. This study provides important novel insights into the mechanisms of UNC-108 recruitment to target membranes, but work is now needed to identify the effectors of UNC-108 that promote phagosome maturation. The current data suggest that a major function of UNC-108 is to recruit lysosomes to the phagosome, a function shared with RAB-14, and genetic experiments suggest they act in parallel. Whether this represents two separate pathways with overlapping functions, or if they share common effectors, remains to be determined.

Some insights into these questions may come from studies of the human GOP-1 homologue, CLEC16A. CLEC16A shares close structural similarity with GOP-1, and Yin et al. (2017) show that CLEC16A can complement *gop-1* mutants, indicating it is likely to interact with Rabs and exhibit GEF activity, although this has not been demonstrated in mammals. Polymorphisms in CLEC16A are associated with a number of autoimmune diseases, including type I diabetes, multiple sclerosis, and rheumatoid arthritis. Intriguingly, these diseases have all been linked to defects in removal of dying cells, raising the possibility that CLEC16A plays a similar role to GOP-1. Lending some support to this, CLEC16A and the *Drosophila melanogaster* homology Ema both regulate the maturation of autophagosomes by promoting lysosomal fusion (Kim et al., 2012; Soleimanpour et al., 2014). CLEC16A has also been shown to interact with the HOPS complex and the Rab7 effector RILP (van Luijn et al., 2015), which are involved in phagosome maturation.

To date, CLEC16A has only been shown to play a role in autophagosome dynamics and it is unclear whether it is involved

in apoptotic cell phagocytosis and phagosome maturation. We speculate that a potential process in which CLEC16A's roles in autophagosome and phagosome maturation may intersect is the process of LC3-associated phagocytosis (LAP), where microtubule-associated protein 1 LC3 and other components of the autophagy machinery are recruited to the phagosome (Sanjuan et al., 2007). LAP promotes lysosomal fusion and rapid acidification and degradation of phagocytosed material, similar to canonical autophagy. Disruption of LAP in macrophages causes delayed degradation of apoptotic cells and also results in increased inflammatory cytokine production (Martinez et al., 2011) and development of autoimmunity (Martinez et al., 2016). Furthermore, as we have recently shown in B cells, noncanonical autophagy also participates in endosomal trafficking of TLRs and their ligands in a process similar to LAP, which limits TLR signaling and prevents autoimmunity (Acharya et al., 2016). An interpretation of these findings is that LAP causes termination of TLR signaling by promoting degradation of phagosomal contents, and, when disrupted, TLR–ligand complexes persist and continue to signal. Hence, we speculate that phagosomes and endosomes containing apoptotic material may be hard wired for accelerated maturation to minimize potential inflammatory signaling. However, the relationship between LAP and immune signaling may be more complex, as noncanonical autophagy also promotes TLR signaling to IRF7 (Acharya et al., 2016) and type I interferon (Henault et al., 2012). It is therefore tempting to speculate that the association of CLEC16A polymorphisms with autoimmunity reflects a potential role of CLEC16A in LAP-mediated trafficking of self-antigens and, given the pleomorphic roles of LAP in different cell types, it will be important to understand the cell-specific effects of CLEC16A on these processes. Additionally, LAP has been shown to occur in *C. elegans* during clearance of midbodies, which occurs through a pathway similar to apoptotic cell uptake, raising the possibility that GOP-1 also interacts with autophagy components in a similar manner to CLEC16A.

Overall, these findings underscore the critical role for coordination of intracellular events during the complex journey that phagosomes and endosomes make through the cell. Notably, delays or deviations in this process can lead to profound consequences, providing a new context for the ancient proverb "many a slip 'twixt the cup and the lip."

Acknowledgments

Work in A. Lacy-Hulbert's and L.M. Stuart's laboratories is funded by National Institutes of Health grants R33AI102266, R21AI1193411, and RO1GM102482. The opinions expressed within do not necessarily represent those of the Bill and Melinda Gates Foundation.

The authors declare no competing financial interests.

References

Acharya, M., A. Sokolovska, J.M. Tam, K.L. Conway, C. Stefani, F. Raso, S. Mukhopadhyay, M. Feliu, E. Paul, J. Savill, et al. 2016. αv Integrins combine with LC3 and atg5 to regulate Toll-like receptor signalling in B cells. *Nat. Commun.* 7:10917. http://dx.doi.org/10.1038/ncomms10917

Henault, J., J. Martinez, J.M. Riggs, J. Tian, P. Mehta, L. Clarke, M. Sasai, E. Latz, M.M. Brinkmann, A. Iwasaki, et al. 2012. Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. *Immunity.* 37:986–997. http://dx.doi.org/10.1016/j.immuni.2012.09.014

Kim, S., S.A. Naylor, and A. DiAntonio. 2012. *Drosophila* Golgi membrane protein Ema promotes autophagosomal growth and function. *Proc. Natl. Acad. Sci. USA.* 109:E1072–E1081. http://dx.doi.org/10.1073/pnas.1120320109

Martinez, J., J. Almendinger, A. Oberst, R. Ness, C.P. Dillon, P. Fitzgerald, M.O. Hengartner, and D.R. Green. 2011. Microtubule-associated protein 1 light chain 3 α (LC3)-associated phagocytosis is required for the efficient clearance of dead cells. *Proc. Natl. Acad. Sci. USA.* 108:17396–17401. http://dx.doi.org/10.1073/pnas.1113421108

Martinez, J., L.D. Cunha, S. Park, M. Yang, Q. Lu, R. Orchard, Q.-Z. Li, M. Yan, L. Janke, C. Guy, et al. 2016. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. *Nature.* 533:115–119. http://dx.doi.org/10.1038/nature17950

Sanjuan, M.A., C.P. Dillon, S.W.G. Tait, S. Moshiach, F. Dorsey, S. Connell, M. Komatsu, K. Tanaka, J.L. Cleveland, S. Withoff, and D.R. Green. 2007. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature.* 450:1253–1257. http://dx.doi.org/10.1038/nature06421

Soleimanpour, S.A., A. Gupta, M. Bakay, A.M. Ferrari, D.N. Groff, J. Fadista, L.A. Spruce, J.A. Kushner, L. Groop, S.H. Seeholzer, et al. 2014. The diabetes susceptibility gene Clec16a regulates mitophagy. *Cell.* 157:1577–1590. http://dx.doi.org/10.1016/j.cell.2014.05.016

van Luijn, M.M., K.L. Kreft, M.L. Jongsma, S.W. Mes, A.F. Wierenga-Wolf, M. van Meurs, M.-J. Melief, R. der Kant, L. Janssen, H. Janssen, et al. 2015. Multiple sclerosis-associated CLEC16A controls HLA class II expression via late endosome biogenesis. *Brain.* 138:1531–1547. http://dx.doi.org/10.1093/brain/awv080

Yin, J., Y. Huang, P. Guo, S. Hu, S. Yoshina, N. Xuan, Q. Gan, S. Mitani, C. Yang, and X. Wang. 2017. GOP-1 promotes apoptotic cell degradation by activating the small GTPase Rab2 in *C. elegans*. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201610001