

DepHining membrane identity

Gerald R.V. Hammond

Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA

How do organelles coordinate their unique molecular identities between their cytosolic-facing surface membranes and their interior? In this issue, Nauffer et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201702179>) discover an intriguing link between phagosome acidification and lipid signposts on their outer membrane.

Membrane-bound organelles that define eukaryotic cells have their form and function defined by their unique protein machineries. However, this intricate organization poses a logistical challenge for cells: how to acquire and maintain these unique molecular repertoires. This is an especially tall order in the secretory and endocytic pathways, where organelles continuously exchange membranes and proteins. In fact, two core mechanisms are used to provide physical landmarks for individual organelles. First, the lumen of these compartments has a unique pH that can activate and deactivate proteins depending on whether they are in the correct organelle. Second, unique molecular signposts define the outer membrane, permitting cytosolic proteins to be recruited to the right compartment. These signposts often take the form of specific proteins, classically Rab-family GTPases (Pfeffer, 2017). However, membrane lipids are also crucial here; specifically, the phosphoinositides have a key role. These lipids contain an inositol head group with three hydroxyl groups that can be decorated by up to three phosphate groups, arranged in any of seven possible configurations by large families of lipid kinases and phosphatases. In this way, they can serve as specific and rapidly modifiable molecular signposts to direct recruitment and activation of specific proteins as membranes transit through the endocytic pathway (Schink et al., 2016). Until now, it has not been clear how these and “outer” identities are coordinated with the “inner” pH-defined identity. In this issue, Nauffer et al. uncover an intriguing link between these defining properties, showing that luminal pH controls the accumulation of an inositol lipid, phosphatidylinositol-3-phosphate (PI(3)P), on the outer surface of endo/lysosomes (Nauffer et al., 2018).

The team was studying phagocytosis, the process by which macrophages and other cells clear pathogens and debris from the body by capturing the particle in a membrane-bound sack, or phagosome. This phagosome fuses with early and then late endosomal compartments, successively acquiring their identity, before eventually fusing with lysosomes, leading to degradation of the internalized body (Bohdanowicz and Grinstein, 2013). In this regard, phagocytosis could be thought of as the classical endocytic pathway writ large.

Phagocytosis has been extensively characterized, primarily by studying uptake of small bodies—rod-shaped bacteria, red blood cells, and even opsonized latex beads. However, Terebiznik’s team has been studying uptake of more irregular shaped bodies, particularly long, filamentous bacteria such as *Legionella* that are often much longer than the phagocyte engulfing it (Prashar et al., 2013). These cells internalize the bacteria into long tube-shaped phagosomes, like sucking up a strand of spaghetti. This process takes much longer to complete and generates an organelle many tens of micrometers in length. It was in studying the maturation of these tubular phagosomes that the authors made their surprising discovery.

As the bacterium is drawn inside the cell, the phagocytic membrane acquires early and then late endosomal markers by fusing with these organelles, as with more conventional particles that are phagocytosed. However, the long temporal and spatial scales mean these processes happen concurrently along the length of the tube. Nauffer et al. (2018) found that regions close to the plasma membrane acquire early endosomal markers, whereas more distal regions that were internalized earlier acquire late endo/lysosomal markers (Nauffer et al., 2018). Intriguingly, one cardinal feature of early endosomes, PI(3)P, was retained along much longer stretches of this tubule and was found coincident with late endosomal and lysosomal markers, which is not the case with smaller particles. In fact, PI(3)P only disappeared when these distal regions began to acidify.

This pool of PI(3)P was depleted by treating cells with Vps34-selective inhibitors, indicating its synthesis by this canonical endosomal phosphoinositide 3-kinase. Use of other kinase-selective compounds revealed that clearance of PI(3)P relied on the activity of another lipid kinase, PIKfyve, that converts it to PI(3,5)P₂. Preventing acidification of the phagosome lumen with weak bases or vacuolar ATPase inhibitors causes PI(3)P to persist even longer, whereas forced acidification drives PI(3)P depletion. This appears to be because of the pH-sensitive association of the Vps34 complex with the membrane, which dissociates when the cytosol is acidified. Intriguingly, similar manipulations on nonphagocytosing cells revealed a similar mechanism at play on conventional endosomes. So, it seems that luminal pH can control lipid synthesis on the outside of the endosome, revealing for the first time how these properties might be coordinated.

Interestingly, this is not the first link between endosomal lipids and pH, though the link had previously been made the other way around. The Kane laboratory discovered that

Correspondence to Gerald R.V. Hammond: g Hammond@pitt.edu

© 2018 Hammond This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons license (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).



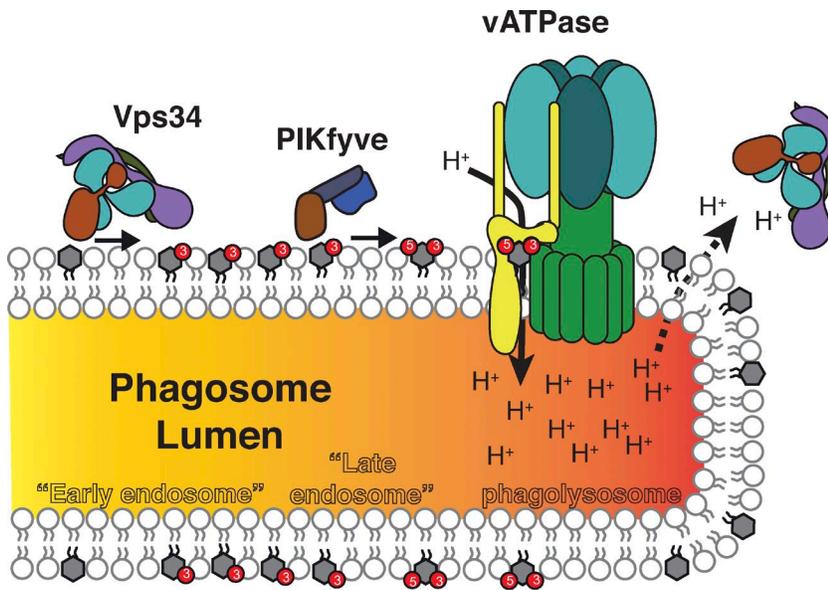


Figure 1. Coordinating lumen and surface phagosome identity. The lipid kinase Vps34 generates PI(3)P on the outer leaflet of the phagosome. As the compartment matures, activation of the vATPase acidifies the lumen. Leakage of protons causes dissociation of Vps34 from the membrane, cutting off PI(3)P synthesis. A kinase necessary for PI(3)P depletion, PIKfyve, generates PI(3,5)P₂ that also activates the vATPase.

the vacuolar ATPase is activated by another inositol lipid, phosphatidylinositol 3,5-bisphosphate (PI(3,5)P₂; Li et al., 2014). Interestingly, the kinase that makes the lipid, PIKfyve, is the very same required for depletion of PI(3)P during phagocytosis (Naufer et al., 2018). We can therefore envisage a model whereby activation of PIKfyve (which is recruited by PI(3)P) activates acidification, leading to the dissociation of Vps34 and the decline of PI(3)P levels (Fig. 1). Notably, PI(3)P is far more abundant in cells than PI(3,5)P₂ (Zolov et al., 2012), so presumably PI(3)P may also be degraded by phosphatases. In this case, blocking of PIKfyve would be expected not just to retain PI(3)P but also to prevent organelle acidification.

Many questions remain as to exactly how this process works. How luminal pH is sensed by the Vps34 complex leading to dissociation is still a mystery. Presumably, proton leak from the acidifying organelle is the trigger, though how this causes unbinding of the kinase is not clear. Although Naufer et al. (2018) were able to determine that it is not through an obvious change in phosphorylation of Vps34, future research will clarify whether pH is sensed by a direct conformational change in the Vps34 complex or is transduced by another pH-regulated factor (a pH-gated calcium channel, perhaps). Also, what happens to other phagosomal lipids in this system is up for debate. Acquisition of another phosphoinositide, PI(4)P, has recently been shown to be required for maturation of phagolysosomes (Levin et al., 2017), and this lipid can also activate vacuolar ATPase subunits (Banerjee and Kane, 2017). It seems possible that coordination of organelle pH and surface lipid content may be a more general phenomenon.

For now, the elegant experiments of Naufer et al. (2018) have uncovered an unexpected link between two defining features of organelle identity. This seems set to become a crucial new concept in our understanding of subcellular organization.

Acknowledgments

G.R.V. Hammond is supported by National Institutes of Health grant 1R35GM119412-01.

The author declares no competing financial interests.

References

- Banerjee, S., and P.M. Kane. 2017. Direct interaction of the Golgi V-ATPase a-subunit isoform with PI(4)P drives localization of Golgi V-ATPases in yeast. *Mol. Biol. Cell.* 28:2518–2530. <https://doi.org/10.1091/mbc.E17-05-0316>
- Bohdanowicz, M., and S. Grinstein. 2013. Role of phospholipids in endocytosis, phagocytosis, and macropinocytosis. *Physiol. Rev.* 93:69–106. <https://doi.org/10.1152/physrev.00002.2012>
- Levin, R., G.R. Hammond, T. Balla, P. De Camilli, G.D. Fair, and S. Grinstein. 2017. Multiphasic dynamics of phosphatidylinositol 4-phosphate during phagocytosis. *Mol. Biol. Cell.* 28:128–140. <https://doi.org/10.1091/mbc.E16-06-0451>
- Li, S.C., T.T. Diakov, T. Xu, M. Tarsio, W. Zhu, S. Couoh-Cardel, L.S. Weisman, and P.M. Kane. 2014. The signaling lipid PI(3,5)P₂ stabilizes V₁-V(o) sector interactions and activates the V-ATPase. *Mol. Biol. Cell.* 25:1251–1262. <https://doi.org/10.1091/mbc.E13-10-0563>
- Naufer, A., V.E.B. Hipolito, S. Ganesan, A. Prashar, V. Zaremborg, R.J. Botelho, and M.R. Terebiznik. 2018. pH of endophagosomes controls association of their membranes with Vps34 and PtdIns(3)P levels. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201702179>
- Pfeffer, S.R. 2017. Rab GTPases: master regulators that establish the secretory and endocytic pathways. *Mol. Biol. Cell.* 28:712–715. <https://doi.org/10.1091/mbc.E16-10-0737>
- Prashar, A., S. Bhatia, D. Gigliozzi, T. Martin, C. Duncan, C. Guyard, and M.R. Terebiznik. 2013. Filamentous morphology of bacteria delays the timing of phagosome morphogenesis in macrophages. *J. Cell Biol.* 203:1081–1097. <https://doi.org/10.1083/jcb.201304095>
- Schink, K.O., K.W. Tan, and H. Stenmark. 2016. Phosphoinositides in control of membrane dynamics. *Annu. Rev. Cell Dev. Biol.* 32:143–171. <https://doi.org/10.1146/annurev-cellbio-111315-125349>
- Zolov, S.N., D. Bridges, Y. Zhang, W.-W. Lee, E. Riehle, R. Verma, G.M. Lenk, K. Converso-Baran, T. Weide, R.L. Albin, et al. 2012. In vivo, PIKfyve generates PI(3,5)P₂, which serves as both a signaling lipid and the major precursor for PI5P. *Proc. Natl. Acad. Sci. USA.* 109:17472–17477. <https://doi.org/10.1073/pnas.1203106109>