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SPOTLIGHT

ER–endosome contacts master the ins and outs of secretory endosomes

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What defines whether an endosome follows the degradative pathway or fuses with the plasma membrane to release exosomes? In this issue of *JCB*, Fredrik Verweij and colleagues (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202112032) demonstrate how secretory endosomes are guided by ER–endosome contacts to take a cellular detour and several identity transitions for efficient exosome release.

The endocytic pathway is not solely a route for degradation of internalized cargo. Endosomes come in many flavors and have distinct functions in the cell, such as signaling platforms, recycling of cargo, plasma membrane repair, lipid and ion exchange, and protrusion formation. To be able to assert their different roles, endosomes display diverse shapes and molecular identities. Whereas cargo destined for lysosomal degradation is sorted into intraluminal vesicles of spherical multivesicular endosomes (MVEs), recycling occurs from endosomal tubules (1, 2). Endosomes vary in their luminal pH, show distinct subcellular localizations, and contain specific proteins and lipids, which allows classifying them into various subpopulations. The presence of phosphoinositides and small GTPases enables the recruitment of effector molecules (3, 4). These can be proteins that mediate endosome fusion, or adaptor proteins connecting the vesicle to actin or microtubule motors for directed transport. Importantly, the ER controls many of these features by making non-fusogenic close contact sites (10–30 nm) with endosomes (5).

The majority of MVEs fuse with lysosomes to degrade their cargo. Some MVEs, however, fuse with the plasma membrane (PM) and release their intraluminal vesicles to the extracellular environment as exosomes (6). Exosomes (50–150 nm) contain proteins, lipids, and RNA and have the ability to communicate with cells in an autocrine and paracrine fashion. Body fluids such as blood and urine contain exosomes, making them useful as biomarkers in cancer.

What defines whether an MVE will fuse with lysosomes or with the plasma membrane? Do the secretory MVEs have a distinct identity? Does ER regulate exosome secretion? By use of their single-cell, live imaging approach to measure exosome release (7), Verweij and colleagues (8) set out to find the answers (Fig. 1).

They fused pH sensitive fluorescent tags (pHuji or pHluorin) to CD63, a protein abundant in MVEs and lysosomes. The fluorescence is quenched in acidic endosomes and lysosomes, but visible as a fluorescent flash when exposed to the neutral extracellular pH upon fusion of MVEs with the PM. By comparing the properties of the MVEs at the time of PM fusion with intracellular MVEs and lysosomes, they found that these had distinct identities. In contrast to lysosomes, the secretory MVEs did not contain the lysosomal hydrolase Cathepsin B and had only trace amounts of the lysosomal protein LAMP1. They lacked classical markers for early, late, or recycling endosomes and rather contained RAB27A and B, known for their role in the fusion of secretory lysosomes and melanosomes with the PM. Indeed, overexpression of RAB27A increased fusion activity, whereas a dominant-negative RAB27A-DN inhibited exosome secretion. They concluded that secretory endosomes represent a subpopulation of MVEs at a prelysosomal stage.

RAB7A has been implicated in exosome release and Verweij and colleagues, strengthen this notion. How could RAB7A promote exosome release, as secretory MVEs do not contain RAB7A? To answer this, they investigated what happens to the MVEs along their journey to the PM. Since ER is a master regulator of endosome function, and many ER–endosome contact sites depend on RAB7A, they asked if ER–endosome contact sites were important for exosome secretion. Indeed, siRNA mediated knock down of two RAB7A dependent contact site proteins, Protrudin or ORP1L, significantly reduced exosome secretion. Whereas Protrudin promotes kinesin-1-mediated transport of RAB7A-positive late endosomes along microtubules to the PM, ORP1L regulates dynein-mediated minus-end transport via the RAB7A effector RILP (5, 9). The authors decided to take a closer look at the function of ORP1L in exosome release.

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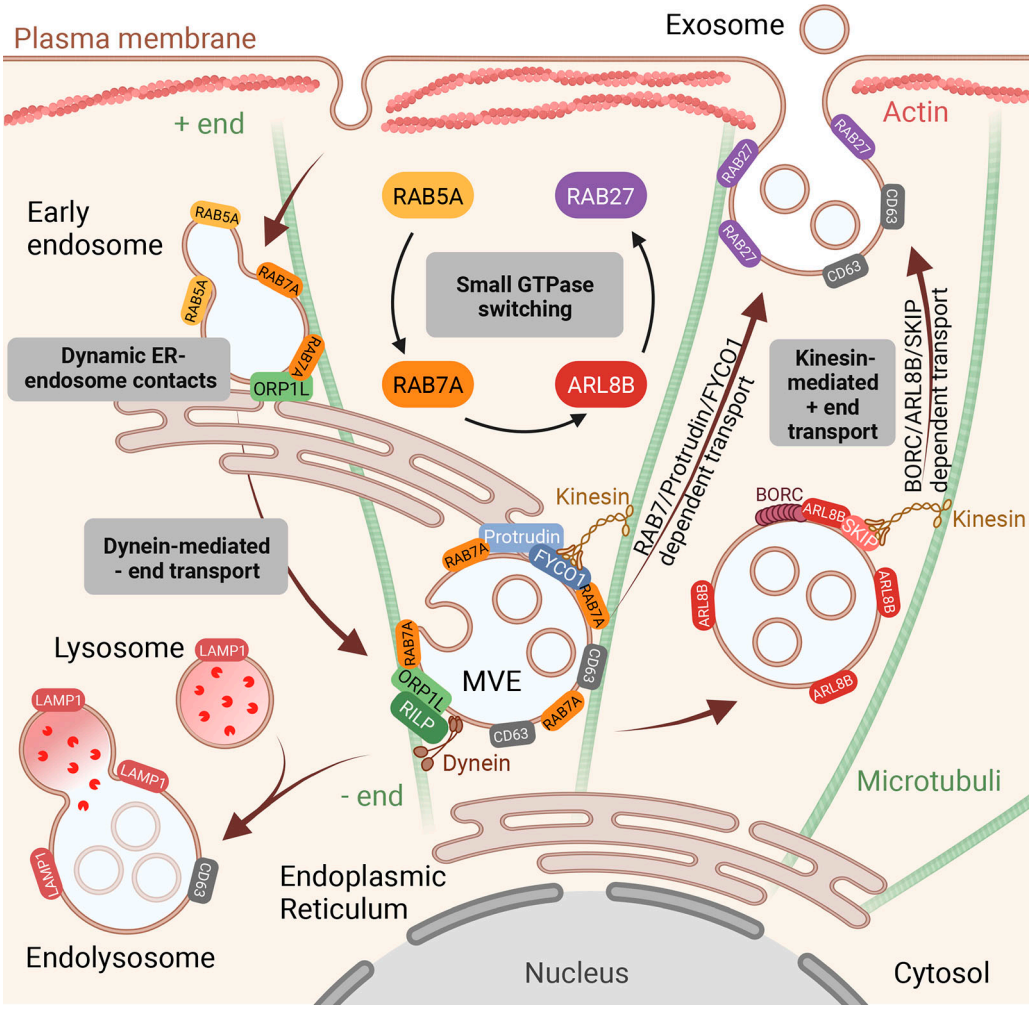

Figure 1. **Dynamic ER–endosome contact sites in exosome secretion.** As endosomes mature, they form intraluminal vesicles and change their GTPase identity from RAB5A to RAB7A. This enables the formation of dynamic ER–endosome contacts via the RAB7A-binding proteins Protrudin or ORP1L. Protrudin mediates kinesin-dependent transport of RAB7A-positive MVEs along microtubules toward the PM, stimulating exosome release. ORP1L dissociation from the ER enables RILP-dependent dynein-mediated transport of MVEs towards the cell center. This facilitates the transition from RAB7A to ARL8B, and subsequent BORC/ARL8B/SKIP/kinesin-dependent MVE transport toward the PM. ARL8B promotes the recruitment of RAB27, involved in actin and microtubule transport across the cell cortex leading to exosome secretion. It is unknown whether Protrudin-mediated exosome secretion depends on GTPase switching before PM fusion.

ORP1L is an endosomal cholesterol sensor. In its cholesterol-free open conformation, an FFAT motif is exposed, mediating interaction with the ER proteins VAPA/B. In its cholesterol-bound closed conformation, ORP1L detaches from the ER, allowing its endosomal interaction partner RILP to engage with dynein (10). They studied the role of ORP1L in exosome secretion by using different mutants of ORP1L that mimic these two conformations. ORP1L in its open, ER-bound form, led to increased ER–endosome contacts, dispersed endosomes with low motility and reduced exosome release. In its closed conformation, ORP1L promoted perinuclear transport of the endosomes, and this surprisingly correlated with increased fusion activity with the PM. A closer look revealed high motility of endosomes moving in and out of the perinuclear endosomal cloud. Could perinuclear translocation of endosomes promote exosome secretion? Yes; indeed, they found that overexpression of RILP promoted perinuclear translocation and exosome release, whereas overexpression of dominant-negative constructs of RILP or dynein inhibited perinuclear endosome localization and exosome release.

How could perinuclear localization of endosomes promote fusion of MVEs with the PM? Kinesin-1 mediates transport of endosomes along microtubules toward the PM and attaches to endosomes by two different mechanisms. One depends on Protrudin and RAB7A that engages the kinesin-1 adaptor FYCO1, as mentioned above. The other depends on BORC, the small GTPase ARL8B, and its effector, the kinesin-1 adaptor SKIP (9). Verweij and colleagues observed that the perinuclear endosomes were devoid of RAB7A and rather contained ARL8B—and that exosome release was in fact dependent on ARL8B. Moreover, overexpression of SKIP increased exosome secretion, suggesting a role for the BORC/ARL8B/SKIP-dependent endosome translocation pathway in exosome secretion from perinuclear MVEs.

In addition to engaging kinesin-1, SKIP also promotes the switch from RAB7A to ARL8B (11). It is plausible that the

perinuclear localization of endosomes, mediated by RAB7A/RILP, facilitates this GTPase switch. However, this still awaits confirmation via direct mechanistic evidence.

The presence of RAB27 at the site of fusion suggested that yet another GTPase switch happens before exosome release. The authors observed a partial overlap between ARL8B and RAB27A, and overexpression of ARL8B increased the amount of RAB27A positive compartments, which were lost upon ARL8B depletion. Expectantly, future studies will shed light on the mechanism of this GTPase transition.

So what could be the role of ER in this multistep process leading to exosome release? The most straightforward explanation would be that Protrudin-mediated ER–endosome contact sites stimulate secretion directly, whereas ORP1L-mediated ER–endosome contacts inhibit exosome fusion by preventing transport of endosomes through the perinuclear path. The results from the present study unexpectedly show that ORP1L promotes exosome release: A mutant of ORP1L, unable to interact with VAP in the ER, inhibited fusion of MVEs with the PM, and ORP1L was required for RAB27A recruitment. Verweij and colleagues conclude that dynamic ER–endosome contact sites are required for successful exosome release. Exactly how the ER promotes this multistep pathway awaits further experimentation. For now, the current study demonstrates that a detour can bring secretory endosomes faster to their destination.

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