# ESCRTing Membrane Deformation

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**The ESCRT-III complex mediates membrane budding away from the cytosol in endosome biogenesis, cytokinesis, and viral budding. In this issue, Saksena et al. (2009) use an elegant fluorescence-based approach to define the sequential activation, recruitment, and disassembly of ESCRT-III subunits during membrane involution in vitro.**

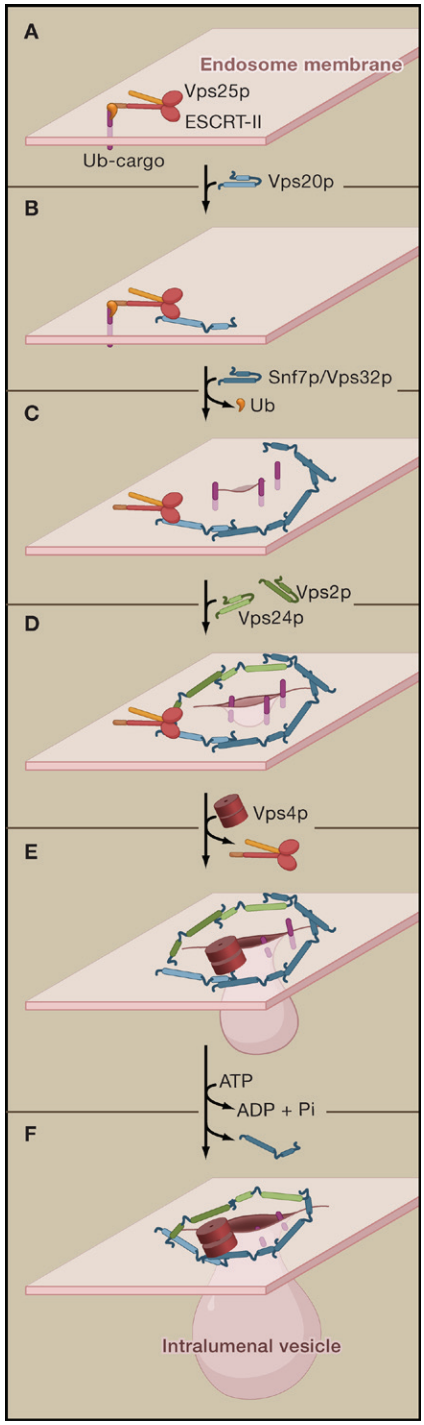
Intracellular membrane trafficking fundamentally involves vesicles that bud from various membranes to be transported elsewhere within the endomembrane system of the eukaryotic cell. Even though most membrane budding events, such as the formation of endocytic vesicles from the plasma membrane, occur toward the cytosol, a few membrane budding processes actually occur with the opposite topology, away from the cytosol. Although the latter include seemingly diverse processes such as the biogenesis of multivesicular endosomes, cytokinesis, and budding of enveloped viruses, such events are mediated by the same machinery, called ESCRT (endosomal sorting complex required for transport). Four separate ESCRT complexes have been described: ESCRT-0, -I, -II, and -III. In contrast to the first three ESCRT complexes, the four subunits of ESCRT-III remain monomeric in cytosol prior to their assembly on endosomes, but the mechanisms that trigger their activation and assembly on membranes are not known in detail. In this issue of *Cell*, Saksena et al. (2009) attempt to solve this puzzle by using a fluorescence spectroscopy-based technique that makes it possible to investigate conformational changes of membrane associated proteins in vitro.

Components of the ESCRT machinery were originally identified and characterized in mutant strains of yeast deficient in vacuolar protein sorting (*vps*) that fail to sort ubiquitinated cargoes into the lumen of the vacuole and that contain aberrant multilamellar endosomes devoid of intralumenal vesicles (Katzmann et al., 2002). Current evidence suggests that the key role of ESCRT-0, -I, and -II, which contain ubiquitin-binding subunits, is to recognize and sequester ubiquitinated proteins in the endosome membrane. In contrast, ESCRT-III, which recruits deubiquitinating enzymes, appears to be responsible for membrane budding in cytokinesis, viral budding, and the biogenesis of intralumenal vesicles. This activity also requires the Vps4p ATPase, which is crucial for the disassembly of ESCRT-III oligomers on membranes.

In order to study the assembly of ESCRT-III on liposome membranes, Saksena et al. covalently attached the fluorescent dye 7-nitrobenzo-2-oxa-1,3-diazole (NBD) to the yeast ESCRT-III subunits Snf7p/Vps32p and Vps20p, which constitute the membrane-proximal subcomplex of ESCRT-III. This dye exhibits higher emission spectra when translocating from an aqueous environment (such as at the surface of soluble protein) to nonaqueous environment (such as the interior of a protein complex or membrane). By monitoring the emission spectra before, during, and after addition of relevant NBD-labeled ESCRT components to liposome membranes, it was possible to follow sequential changes in the conformations of Snf7p and Vps20p. Vps20p was previously found to mediate the interaction of ESCRT-III with ESCRT-II (Katzmann et al., 2002), but ambiguous results of possible interactions between Snf7p and Vps25p in ESCRT-II have also been reported. The present study uses fluorescence resonance energy transfer (FRET) to show that Snf7p and Vps25p do not interact in solution or on membranes. Instead, Vps25p interacts with Vps20p to trigger ESCRT-III activation and recruitment (Figure 1A).

In cytosol, Snf7p appears monomeric, and the closed inactive state is probably preserved by an autoinhibitory interaction between the $\alpha$5 helix in the C terminus and the core $\alpha$2 helix (Figure 1B) (Muziol et al., 2006; Zamborlini et al., 2006). Given that all the ESCRT-III subunits contain fairly similar protein structures, it seems likely that this interaction constitutes a common mechanism to keep ESCRT-III subunits monomeric in the cytosol (Lata et al., 2008; Muziol et al., 2006; Shim et al., 2007; Zamborlini et al., 2006). Thus, formation of ESCRT-III complexes on endosomes appears to require conformational changes, most likely triggered by Vps25p and then propagated sequentially by activated ESCRT-III subunits. According to the data presented in this issue, Vps25p binding opens up the closed, monomeric form of Vps20p, and exposure of the $\alpha$2-loop makes homo- and heterodimerization possible (Figure 1B). Next, Vps20p recruits Snf7p to the endosomal membrane and initiates nucleation of Snf7p oligomers resulting in long filaments (Figure 1C). So far it has been unknown how deubiquitinated cargo can be sequestered by ESCRT-III, and it is hypothesized by the authors that the Snf7p filaments form circular fences that function to concentrate cargo even after its deubiquitination.

The endosomal sorting of ubiquitinated cargo culminates with sequestestration of deubiquitinated cargo into intralumenal vesicles. Interestingly, Saksena and coworkers observed by




electron microscopy membrane deformation in the context of inward membrane invagination on liposomes incubated with ESCRT-III subunits. The observed membrane deformation in vitro is quite similar to formation of intralumenal vesicles of multivesicular endosomes, and it is reasonable to assume that ESCRT-III alone is sufficient to sequester accumulated cargo. Importantly, incubation of liposomes with excessive Snf7p compared to Vps20p, Vps24p, and Vps2p was required for invagination to occur in vitro. This is in accordance with previous reports demonstrating a higher percentage of Snf7p located on membranes in comparison to the other ESCRT-III subunits (Teis et al., 2008). Collectively these data strongly indicate a functional role of long Snf7 filaments growing from a Vps20p-nucleating site on the membrane.

The new in vitro studies, in conjunction with biochemical analyses of intact yeast cells (Teis et al., 2008), provide a model for how ESCRT-III mediates endosomal cargo sorting and biogenesis of multivesicular endosomes (Figure 1) and offer a possible explanation to the paradox that ESCRT-III appears able to sort cargoes into intralumenal vesicles after their ubiquitin tag has been removed. The length of the Snf7p oligomers assembling on endosome membranes appears to be about ten copies (Teis et al., 2008), and it remains to be established how this optimal length is sensed and maintained. The growth of Snf7p filaments is hypothesized to end upon engagement of the Vps24p-Vps2p subcomplex, which could function as a cap at the growing end to prohibit further oligomerization (Figure 1D). Such capping could either be a stochastic process determined by the relative concentrations of Snf7p, Vps24p, and Vps2p or be defined by structural constraints. Both Vps24p and Snf7p have been shown to make long, hollow filaments in vitro (Ghazi-Tabatabai et al., 2008), and it is still not clear whether one or several Vps24p-Vps2p subcomplexes cap the growing Snf7p end. An additional function of this Vps24p-Vps2p cap is probably to recruit Vps4p via binding to Vps2p (Figure 1E). ATP hydrolysis might provide the required energy for Vps4p to release Snf7p molecules from the nucleating end. Assuming that capped Snf7p filaments assemble on endosomes to form shorter versions of the circular arrays observed in the plasma membrane of mammalian cells overexpressing Snf7 alone (Hanson et al., 2008), the disassembly process might shrink the ring. This could ultimately drive fission of the invaginated membrane structure into a vesicle (Figure 1F).

Since the first characterizations of ESCRTs at the beginning of this millennium, there has been a very rapid progress in our understanding of how these complexes mediate protein sorting and membrane deformation. Building on data obtained from biochemistry, cell biology, and structural biology, the paper by Saksena et al. goes one step further by defining how ESCRT-III subunits are regulated by each other and by membrane interactions. Although we still do not know exactly how ESCRT-III functions to deform membranes and how ESCRT-III subunits escape being included into intralumenal vesicles, the new data provide an excellent framework for further experimentation in vitro and in vivo. Considering that ESCRTs control vital cellular processes and are implicated in resistance to cancer, infections, and neurodegeneration, a further understanding of how they work is certainly of interest for future applications in biotechnology and in the development of new pharmaceuticals.

**Figure 1. Step-by-Step Assembly of the ESCRT-III Complex**

Ordered assembly and disassembly of the ESCRT-III complex to mediate cargo sorting and invagination of endosome membranes (Saksena et al., 2009). Assembly starts with recruitment of the ESCRT-III subunit Vps20p by the ESCRT-II subunit Vps25p on endosomes (A), which causes activation of Vps20p through release of an autoinhibitory interaction (B). The activated Vps20p in turn binds Snf7p/Vps32p and nucleates Snf7p monomers to oligomerize into circular filaments that mediate cargo entrapment and membrane invagination (C). Upon binding of Vps24p-Vps2p, oligomerization terminates (D) and Vps4 is recruited (E) and facilitates disassembly of ESCRT-III by releasing Snf7p molecules (F). The Vps4p-mediated removal of Snf7p monomers at the end of the filament might cause its constriction, ultimately leading to membrane abscission. Each individual interaction facilitates specific conformational changes opening up and closing protein structures allowing only specific partners to interact. Cumulatively, these changes in protein conformations lead to the recruitment of a specific sequence of factors, thereby driving and controlling each step along the pathway for cargo sorting and membrane deformation. ESCRT-III-mediated membrane deformation in cytokinesis and viral budding is likely to be equivalent to that proposed for the biogenesis of intralumenal vesicles, with the exception that the initial recruitment and activation of Vps20 is mediated by other components (Tanaka et al., 2008).

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# Who Benefits from Granulomas, Mycobacteria or Host?


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**By investigating host-pathogen interactions in zebrafish using intravital imaging, Davis and Ramakrishnan (2009) provide evidence that aggregates of immune cells known as granulomas, long thought to constrain mycobacterial infection, may instead facilitate its spread.**


In criminal cases, the concept of *cui bono* ("who benefits") is often invoked to characterize those who stood to gain from a crime. When the same principle of inquiry is applied to the complexities of host-pathogen interactions, the results can be surprising. Such is the case of Davis and Ramakrishnan (2009) who report in this issue the use of quantitative intravital imaging to study the initial steps of *Mycobacterium marinum* infection in a zebrafish model of tuberculosis. Their findings suggest that organized aggregates of immune cells called granulomas, which accumulate at sites of tuberculosis infection, are not necessarily beneficial to the host, at least in the early stages of infection. Instead, they show that granulomas may act as unwitting accomplices in the spread of *M. marinum* infection.

*Mycobacterium tuberculosis*, which causes tuberculosis in humans, is a highly successful bacterium that infects macrophages and dendritic cells and is hence well positioned to subvert and exploit host immune responses. *M. tuberculosis* poses a quandary for the immune system: although adaptive ($CD4^+$ and $CD8^+$ T cell) immunity is essential for control of bacterial growth, it cannot eradicate infection. The result is latent infection—a hallmark of tuberculosis—that persists for the lifetime of the host and reactivates with sufficient frequency to maintain a worldwide epidemic. Therefore, studies of host responses to virulent mycobacteria provide opportunities to identify the limitations of immunity. Understanding the shortcomings of the immune responses to *M. tuberculosis* and the virulence strategies it uses to evade immunity remain essential prerequisites for development of an effective tuberculosis vaccine.

The characteristic lesions of tuberculosis are granulomas, which have long been considered a host defense mechanism for containing persistent pathogens (Adams, 1976; Egen et al., 2008). Granulomas are classically defined as organized collections of macrophages and lymphocytes that form to eliminate foreign substances. Their unique cellular organization led to the belief that granulomas physically "wall off" persisting bacteria while preserving the function of neighboring tissue. An alternative model is that mature granulomas represent a stalemate or equilibrium between host and pathogen, characterized by adaptive immunity sufficient to arrest progressive infection but insufficient to kill the intracellular bacteria. Until recently, investigations of granulomas have been constrained by imaging techniques that required fixed tissue, limiting insights into cellular dynamics and the molecular mechanisms of formation and maintenance of their structure.

Fortunately, recent innovations in intravital imaging have enabled a more complete analysis. Real-time imaging of *M. marinum* infection in transparent zebrafish embryos has demonstrated that granuloma formation by infected macrophages occurs within 3 days of infection and does not require adaptive immunity (Davis et al., 2002). Further work in this model revealed that the cytokine tumor necrosis factor (TNF),