The circuitry of cargo flux in the ESCRT pathway

James H. Hurley and Xuefeng Ren

The endosomal sorting complex required for transport (ESCRT) complexes sort ubiquitinated membrane proteins into multivesicular bodies, which is a key step in the lysosomal degradation pathway. Shields et al. (Shields, S.B., A.J. Oestreich, S. Winistorfer, D. Nguyen, J.A. Payne, D.J. Katzmann, and R. Piper. 2009. J. Cell Biol. 185:213–224) identify a new ubiquitin-binding site in ESCRT-I and provide evidence that the upstream ESCRT-I and -II complexes sort cargo in parallel rather than in series.

Series or parallel? This is the most basic question that can be asked about a circuit, whether electrical or biological. The ESCRT protein complexes sort ubiquitinated transmembrane proteins into multivesicular bodies (MVBs). MVBs are intermediate structures in the endosomal–lysosomal pathway that are formed when portions of the endosomal limiting membrane bud into the lumen of the endosome, forming intraluminal vesicles (ILVs). The ESCRT machinery consists of five multiprotein complexes, ESCRT-0, -I, -II, -III, and Vps4–Vta1 (Saksena et al., 2007; Williams and Urbe, 2007; Hurley, 2008), along with several associated proteins. ESCRT-III carries out the scission of the ILVs from the limiting membrane, and the Vps4–Vta1 complex recycles ESCRT-III (Wollert et al., 2009). Thus, ESCRT-III and Vps4–Vta1 act in series with respect to each other, and both act downstream of the other components. Early models of ESCRT function suggested that the upstream complexes ESCRT-0 through -II also functioned in series, perhaps handing off ubiquitinated cargo from one complex to another in a sort of bucket brigade. However, biological circuits, just like electrical ones, can also act in parallel. In fact, recent evidence indicates that upstream ESCRT complexes act in parallel. In this model, the upstream ESCRTs form a supercomplex that clusters ubiquitinated cargo in preparation for its internalization (Hurley, 2008). Now, new work from Shields et al. (see p. 213 of this issue) provides some of the most compelling evidence yet that the upstream ESCRTs work together in parallel.

The resistance of a series of resistors in a direct current circuit is the direct sum of the resistances of the individual resistors. In contrast, the resistance of a set of resistors in parallel is obtained from the reciprocal of the sum of the reciprocals of the individual resistors. A high resistance placed in series impedes the current through the entire circuit, whereas a high resistance in a parallel circuit has little effect, causing the current to bypass it through lower resistances. Shields et al. (2009) applied this principle (although they do not frame it as such) to the flux of ubiquitinated cargo through the upstream portion of the ESCRT system. By using crystal and nuclear magnetic resonance structures of ESCRT ubiquitin-binding domains in complex with ubiquitin, they were able to generate mutant ESCRT complexes selectively crippled in individual ubiquitin-binding domains. In the course of the study, they deduced the presence of a ubiquitin-binding domain at the C terminus of the ESCRT-I subunit Mvb12. The Mvb12 ubiquitin-binding domain consists of a short stretch of residues that are disordered, at least in the ESCRT-I tetramer structure, and does not conform to any established ubiquitin-binding motif. This discovery highlights how much there is still to be learned about ubiquitin-binding motifs and how many as yet undetected motifs might exist.

Crippling the ubiquitin interaction with the only known ubiquitin-binding domain, the second zinc finger (NZF2), in yeast ESCRT-II had essentially no phenotype. Sorting was maintained even when one of the ubiquitin-binding sites in ESCRT-I, the ubiquitin E2 variant (UEV) domain of the Vps23 subunit, was inactivated. Inactivation of the novel ESCRT-I ubiquitin-binding site in Mvb12 had no phenotype on its own. However, the simultaneous inactivation of all three sites blocked the sorting of most, although not all, cargoes tested. The ESCRT pathway is complex, and a variety of explanations for the cooperative behavior of the ubiquitin-binding domains can be put forward. However, given what we know from biochemical, structural, and other genetic studies (Saksena et al., 2007; Williams and Urbe, 2007; Hurley, 2008), the most reasonable deduction is that ESCRT-I and -II work in parallel with respect to the equivalent of an electrical current in this system, the flux of ubiquitinated cargo.

The new study also sheds light on the mechanisms ESCRT-I uses to bind ubiquitinated cargo. In both yeast and animals, the N-terminal UEV domain of the Vps23 subunit binds to a single ubiquitin moiety (Katzmann et al., 2001; Sundquist et al., 2004).

Correspondence to James H. Hurley: hurley@helix.nih.gov
A second ubiquitin-binding site is now revealed at the C terminus of the Mvb12 subunit of yeast ESCRT-I. This places the two ubiquitin-binding domains in close proximity to each other in three dimensions (Kostelansky et al., 2007). The human MVB12A and B isoforms are very divergent in sequence from yeast Mvb12, and it is not clear whether they bind ubiquitin or not. However, the VPS37A subunit of human ESCRT-I contains a ubiquitin-binding domain at its N terminus (Williams, R.L., personal communication), which is in close spatial proximity to the other ESCRT-I–binding sites. This is also true of the ubiquitin-binding sites on the subunits of the ESCRT-0 complex (Hirano et al., 2006; Ren et al., 2009). Seeing these sites so close together highlights another longstanding issue in the ESCRT and ubiquitin-dependent sorting field: what is the nature of the ubiquitin modification that signals entry into the ESCRT pathway? Early work suggested that monoubiquitin was the key signal, but more recent data suggest that Lys-63 (K63)–linked polyubiquitin may be the predominant signal. Some 50% of the ubiquitin on the EGF receptor, the best studied ESCRT substrate in human cells, is in the form of a K63-linked polymer (Huang et al., 2006). In yeast, both the cell surface transporter Gap1 and the vacuolar hydrolase Cps1 require K63-linked polyubiquitination for entry into MVBs (Andre, B., personal communication). The occurrence of two ubiquitin-binding sites in close spatial proximity in both the Hrs and STAM subunits of ESCRT-0 and in ESCRT-I suggests a mechanism for recognition of the K63 polyubiquitin chain. It also raises the question as to whether ESCRT-II, which is now the only upstream complex to contain only one known ubiquitin-binding domain, contains additional as yet unidentified ubiquitin-binding sites.

How might the concept of a parallel circuit for a ubiquitinated cargo “current” translate into a biochemical mechanism? Yeast ESCRT-I and -II form a high-affinity supercomplex of 1:1 stoichiometry (Gill et al., 2007) that coassembles in vitro on model membranes (Kostelansky et al., 2007). ESCRT-0 and -I also physically interact, although work in our laboratory indicates that the binding is far weaker than for ESCRT-I–II (unpublished data), and Shields et al. (2009) find that the putative ESCRT-I–binding site on ESCRT-0 is not required for function. Thus, there is strong physical evidence in support of an ESCRT-I–II supercomplex as well as evidence for at least a transient ESCRT-0–I interaction. It is plausible, although currently unproven, that all three could simultaneously assemble into a physical complex (Fig. 1, left). The membrane surface area covered by a modeled 1:1:1 supercomplex of ESCRT-0, -I, and -II is of the correct magnitude to give rise to a single ILV in yeast (Hurley, 2008). In animal cells, ILVs are larger, and the supercomplex would need to have more than one copy of each individual complex (or there could be several copies of a 1:1:1 supercomplex). In this model, the supercomplex would fill the role that in more conventional vesicle trafficking systems is served collectively by coat and cargo adaptor proteins (Bonifacino and Glick, 2004). Current schemes envisage that the supercomplex, via ESCRT-II, would initiate the recruitment and assembly of ESCRT-III (Fig. 1, middle; Saksena et al., 2009). As yet unknown factors, which could perhaps include deubiquitinating enzymes, would then trigger the release of the supercomplex from the membrane, leading to the closure of the ESCRT-III array and scission of cargo-containing vesicles into the lumen of the endosome (Fig. 1, right). The budding away from the cytosol that occurs in the MVB pathway is distinct from other vesicular trafficking pathways in cells and thus requires mechanisms that have little precedent in conventional pathways. The synthesis of genetics, biochemistry, and structural analysis in the field, which the work of Shields et al. (2009) exemplifies in miniature, is finally leading to the outlines of a plausible mechanism for this most unusual sorting pathway.

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