Vps13 and Cdc31/centrin: Puzzling partners in membrane traffic

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Yeast Vps13 is a member of a conserved protein family that includes human homologues associated with neurodegenerative and developmental disorders. In this issue, De et al. (2017. J. Cell Biol. https://doi.org/10.1083/jcb.201606078) establish direct roles for Vps13 and its surprising binding partner, the calcium-binding centrin Cdc31, in trans-Golgi network (TGN) to endosome traffic and TGN homotypic fusion.

Isolation of vacuolar protein sorting (vps) mutants using budding yeast opened inroads to the evolutionarily conserved molecular machinery responsible for sorting proteins from the TGN and endosomes to the lysosome-like vacuole. Subsequent characterization of Vps proteins, combined with analysis of trafficking defects in vps mutants, contributed to understanding fundamental principles of protein traffic, including those that govern transport vesicle formation, targeting, and fusion. Despite these advances, functions of several Vps proteins remain undefined. One enigma is Vps13, the founding member of a conserved family that includes four members in humans (Vps13A–D), three of which (A–C) are associated with inherited nervous system disorders (Velayos-Baeza et al., 2004). In this paper, De et al. provide insights into the structure of Vps13 and its role in membrane transport and organelle fusion.

The VPS13 gene family encodes large proteins that share homologous regions at the N and C termini and an internal domain of unknown function (Velayos-Baeza et al., 2004). The C-terminal region contains a putative PH domain (Fidler et al., 2016) but otherwise there are no clear similarities to other proteins. Analyses of yeast Vps13 and homologues in other species present a complex puzzle that hints at broad participation in fundamental principles used for the TGN-PVC assay except the substrate is localized to the TGN rather than the PVC. In this case, the protease gains access to the substrate by direct fusion of the donor and acceptor TGN without vesicle intermediates. In this assay, Vps13 was required in both donor and acceptor fractions, suggesting that Vps13 plays a key role in membrane docking and/or fusion.

A powerful advantage of cell-free transport assays is the opportunity to test for direct function by adding back a purified component to complement defects in extracts from mutant strains. De et al. (2017) used affinity purification strategies to isolate a soluble form of Vps13 from yeast cells engineered to overexpress the protein. Purified Vps13 fully restored both TGN-PVC transport and TGN homotypic fusion when added back to vps13Δ fractions, providing strong evidence that Vps13 directly acts in both processes.

One prior clue to Vps13 function comes from studies of the sporulation defect in vps13Δ cells, which indicate that expansion of the prospore membrane is slowed (Park and Muñoz-Braceras et al., 2015). It is unclear which of these roles rely on common functions and which are a result of specialization of individual family members.

Given the plethora of attributed roles, specific assays are needed to define Vps13 function and build paradigms to test in other systems. De et al. (2017) assessed Vps13 function in two yeast cell-free assays developed to dissect vesicle-mediated transport from the TGN to the prevacuolar compartment (PVC)/late endosome or homotypic fusion of TGN membranes. Such assays can provide direct measures of transport through a pathway without complexities of alternative routes and indirect effects that can occur in vivo. In the TGN-PVC assay, active fractions from semi-permeabilized cells are prepared from two yeast strains, one expressing a TGN-localized protease (donor) and the other expressing a substrate that resides in the PVC (acceptor). Mixing two fractions results in transport of the protease to the PVC and cleavage of the substrate, dependent on factors necessary for transport vesicle formation at the TGN and vesicle fusion to the PVC in vivo. De et al. (2017) tested whether Vps13 was required for TGN-PVC transport by preparing fractions from wild-type or VPS13 deletion (vps13Δ) strains. By this assay, vps13Δ acceptor fractions were completely inactive, indicating that Vps13 is critical at the PVC where vesicle docking and fusion occur. In contrast, Vps13 was partly dispensable in the donor fraction where transport vesicles containing the cargo protease form from the TGN. The TGN homotypic fusion assay is based on the same principles used for the TGN-PVC assay except the substrate is localized to the TGN rather than the PVC. In this case, the protease gains access to the substrate by direct fusion of the donor and acceptor TGN without vesicle intermediates. In this assay, Vps13 was required in both donor and acceptor fractions, suggesting that Vps13 plays a key role in membrane docking and/or fusion.

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Neiman, 2012). Expansion occurs by vesicle fusion to the growing prospore membrane, which requires phospholipase D–mediated production of phosphatidic acid (PA), a phospholipid that can promote membrane fusion. Vps13-deficient cells exhibit reduced levels of PA and its precursors, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate, in the prospore membrane, suggesting a role for Vps13 in regulating these phospholipids. Following up on this clue, De et al. (2017) determined that purified Vps13 binds selectively to several phospholipids in the context of synthetic liposomes, most avidly to PA but also to mono- and diphosphorylated phosphatidylinositol-4-phosphate. Fragments from the conserved N- and C-terminal regions interacted with a subset of the lipids recognized by full-length protein, suggesting multiple lipid-binding sites along Vps13. Although the significance of lipid binding remains to be determined, C- or N-terminal lipid-binding fragments inhibited cell-free TGN-PVC transport, perhaps by interfering with full-length Vps13 present in the assay.

Purified Vps13 also allowed De et al. (2017) to pursue the functional significance of a long-standing observation that Vps13 interacts with the yeast centrosome Cdc31, a component of the spindle pole body (SPB) that serves as the microtubule organizing center in yeast (Kilmartin, 2003). Cdc31 has a well-characterized role in SPB duplication during cell division, but has also been associated with independent processes including cell morphogenesis and integrity (Ivanovska and Rose, 2001). De et al. (2017) found that purified Vps13 was associated with Cdc31, suggesting that they form a stable complex. To assess possible roles in Vps13-mediated transport, strains expressing a collection of temperature-sensitive cdc31 (cdc31-ts) alleles were screened for defective vacuolar protein sorting in vivo. Many, but not all, of the alleles caused defects at the nonpermissive temperature. These alleles harbor single mutations spread throughout Cdc31, including two in the N-terminal region that do not affect SPB duplication or cell morphogenesis/integrity (Ivanovska and Rose, 2001). This provides evidence that Cdc31 acts specifically in vacuolar protein sorting, independently of roles in the other processes. In cell-free TGN-PVC and TGN homotypic fusion assays, fractions from strains expressing a cdc31-ts allele (affecting multiple pathways) displayed defects that mirrored those of vps13Δ fractions. Activity of cdc31-ts fractions was restored in both assays by adding Vps13 purified from wild-type cells but not from cdc31-ts cells. Strikingly, Vps13 purified from cdc31-ts cells was also unable to complement fractions from vps13Δ cells even though the fractions presumably contained normal levels of wild-type Cdc31. Together these findings offer compelling evidence that a complex of Vps13 and active Cdc31 is required for Vps13 function in vesicle-mediated TGN-PVC transport and TGN homotypic fusion.

One final tantalizing finding was made possible by the availability of purified Vps13—the overall architecture as determined by single particle electron microscopy. Vps13 particles have a distinctive shape, with an extended trunk region flanked by a loop at one end and a hook-like structure at the other (Fig. 1). Two types of class averages showed the hook protruding from the trunk in either the same or opposite direction as the loop, suggesting rotational flexibility in the trunk. How Vps13 is arranged in the two particles, where Cdc31 is located, whether the loop can open and close, and how the distinguishing features contribute to Vps13 function are just some of the questions raised by this remarkable shape.

The work of De et al. (2017) has brought parts of the Vps13 puzzle into focus and identified new pieces: cell-free assays suggest a prominent role in docking and/or fusion and a lesser role in vesicle formation, there are multiple sites for binding to phospholipids known to be important in membrane trafficking, association with Cdc31 is necessary for function, and the protein assumes an unusual architecture. The challenge for the future will be to put these pieces together for a more complete picture of Vps13 function.

The newly identified lipid binding properties of Vps13 may be a key to its function, thus it will be important to define the binding sites and test their roles in vitro and in vivo. By analogy to other components of the trafficking machinery, phosphoinositide binding may be important for Vps13 recruitment to the appropriate membrane. Lipid binding could also contribute to activity in docking and/or fusion. For example, sites for different phosphoinositides could provide a way for Vps13 to bridge membranes from two sources, as would occur in TGN-derived vesicle docking to the PVC. It is intriguing that purified Vps13 bound most strongly to PA, which can promote membrane fusion. Notably, the lipids that are preferentially bound by purified Vps13 are
lipid levels needs to be addressed. In sporulating cells, the mechanism by which Vps13 regulates Vps13 interaction with the membrane. In this case, and in sporulating cells, the mechanism by which Vps13 regulates lipid levels needs to be addressed.

Cdc31 association raises other important questions. How does Cdc31 contribute to Vps13 activity? Cdc31 could be a core structural component that helps form/stabilize the unusual structure. It could also regulate Vps13 activity more dynamically. Cdc31 is a calcium-binding protein and so could confer calcium sensitivity to Vps13 activity. Consistent with this idea, cell-free TGN homotypic fusion is blocked by rapid chelation of calcium, suggesting involvement of transient calcium flux (Brickner et al., 2001). However, calcium binding is not required for some Cdc31-mediated activities. Consequently, it will be informative to assess the role of Cdc31 and calcium binding in Vps13 structure and function in TGN fusion, TGN-PVC transport, and prospore membrane formation.

The possibility of roles for lipid binding and regulation, Cdc31 and calcium, and the distinctive structure also apply to Vps13 function at organelle junctions. Multiple lipid binding sites on Vps13 are well-suited for bridging two organelle membranes at a junction and perhaps for facilitating lipid exchange. Vps13 is localized to junctions between organelles that are major repositories of calcium—ER/nuclear envelope, vacuole, and mitochondria—which may, in some yet-to-be-defined way, contribute to calcium regulation of Vps13 via Cdc31.

Considering the findings of De et al. (2017), it appears that Vps13 may commonly act in processes that involve close juxtaposition of membranes, making it tempting to speculate that one core function of Vps13 is to physically connect membranes. This hypothesis may also be useful in guiding models of Vps13 function in other species (Fig. 1). In humans, mutations in three of the four VPS13 genes cause distinct neurodegenerative or developmental disorders: chorea acanthocytosis (VPS13A), Cohen’s syndrome (VPS13B), and autosomal recessive Parkinson’s disease (VPS13C). Relatively little is known about the genes or the molecular and cellular basis of the diseases, yet there are resemblances to yeast Vps13 that share a common core function in bridging membranes. Vps13A has been localized to Golgi and/or vesicles in some cell types and plays a role in maintaining specific phosphoinositide levels (Park et al., 2015). Vps13B is associated with the Golgi complex and is implicated in connecting adjacent membrane stacks in the Golgi ribbon (Seifert et al., 2011). Vps13C partly localizes to mitochondria and plays a role in mitochondrial integrity (Lesage et al., 2016). In a recent study, disease-causing missense mutations in VPS13A, when transferred to yeast Vps13, most frequently caused specific defects in mitochondrial integrity, suggesting that disease might stem in part from mitochondrial dysfunction (Park et al., 2016). However, to date VPS13A has not been detected on mitochondria in human cells, so the significance of the yeast phenotypes for the human disease awaits resolution. As experiments such as these add to the complexity of the Vps13 puzzle, advances in understanding the fundamental properties of Vps13, like those of De et al. (2017), will be critical for piecing together the full picture.

Acknowledgments

The authors apologize to colleagues whose papers are not referenced because of space limitations.

The Payne laboratory is funded by National Institutes of Health (RO1 GM39040).

The authors declare no competing financial interests.

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