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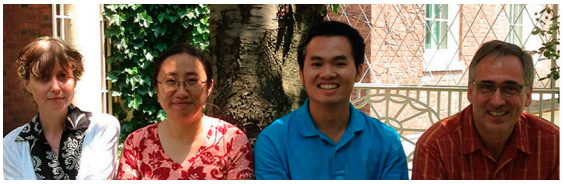
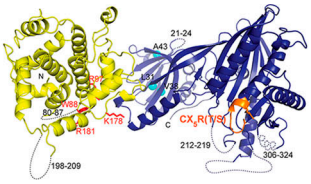
Vps74 gives phosphatase directions

Study reveals how the Sac1 phosphatase removes PtdIns4*P* from early Golgi membranes.

Focal Point





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(Left to right) Karin Reinisch, Yiying Cai, Yongqiang Deng, Christopher Burd, and (not pictured) Florian Horenkamp demonstrate how cells restrict the phosphoinositide PtdIns4*P* to the trans-Golgi network. After determining the crystal structure (right) of the PtdIns4*P*-binding protein Vps74 (yellow) bound to the phosphoinositide phosphatase Sac1 (blue), the researchers identified mutations that specifically inhibit the interaction between these two proteins and examined their effect on PtdIns4*P* distribution and Golgi function. The results suggest that Vps74 acts as a membrane receptor for Sac1, recruiting the phosphatase to hydrolyze PtdIns4*P* in early Golgi compartments such as the medial cisternae and thereby maintaining the identity and function of the trans-Golgi network.

Phosphoinositides have a crucial role in specifying the identity of different cellular compartments. In the Golgi apparatus, for example, phosphatidylinositol 4-phosphate (PtdIns4*P*) is specifically generated at the trans-Golgi network (TGN) by phosphatidylinositol 4-kinase and is removed from other Golgi compartments by the lipid phosphatase Sac1. Sac1 is an integral membrane protein that mainly localizes to the endoplasmic reticulum. Its phosphatase domain is tethered to the membrane by a long unstructured linker, so how it finds its substrate on other organelles such as the Golgi is unclear. Cai et al. reveal that the PtdIns4*P*-binding protein Vps74 acts as a membrane receptor for Sac1, directing the phosphatase to early Golgi membranes to restrict the distribution of PtdIns4*P* and maintain the distinct identities of Golgi cisternae (1).

PtdIns4*P* recruits the budding yeast protein Vps74 (a homologue of mammalian GOLPH3) to the TGN. The protein packages Golgi-resident glycosyltransferases into retrograde transport vesicles that return the enzymes to the cis- and medial-Golgi cisternae (2, 3). "Yeast lacking Vps74 fail to retain these enzymes in the Golgi and instead deliver them to the vacuole where they're degraded," explains Christopher Burd from Yale University School of Medicine in New Haven, Connecticut.

Retrograde vesicles also transport PtdIns4*P* from the TGN to earlier Golgi compartments. In 2012, Burd and colleagues found that Vps74 binds to Sac1 and that deleting either of these two proteins elevated the level of PtdIns4*P* on medial-Golgi membranes (4). "We proposed that Vps74 grabs onto Sac1 and delivers it to the early Golgi so that it can scrub PtdIns4*P* from the membrane," says Burd. Testing this hypothesis was difficult, however, because Vps74 interacts with many different Golgi proteins. Burd and colleagues, led by Yiying Cai and Yongqiang Deng, therefore sought to identify mutations that inhibited Vps74's association with Sac1 without affecting its interactions with other proteins or with PtdIns4*P*.

> **"The disorganization that arises from the spread of PtdIns4*P* has a functional consequence."**

Cai et al. determined the crystal structure of the complex formed between Vps74 and the N-terminal portion of Sac1, which contains both the phosphatase's catalytic domain and an additional, well-conserved, N-terminal subdomain (1). The researchers discovered that Vps74 interacts with the N-terminal subdomain of Sac1 and identified point mutations in Vps74 that specifically disrupted this interaction.

Yeast expressing this Vps74 mutant showed increased PtdIns4*P* levels at the medial Golgi, supporting the idea that Vps74 acts as a membrane receptor for Sac1. Moreover, the mutant yeast cells were less able to retain the mannosyltransferase Kre2 in the medial Golgi; the enzyme was delivered to the vacuole lumen instead. "So the disorganization that arises from the spread of PtdIns4*P* has a functional consequence," says Burd. One possibility is that, when PtdIns4*P* turnover is inhibited, Vps74 gets trapped on the medial Golgi membrane and can't be recycled to the TGN to participate in retrograde transport and enzyme retention.

"Our work highlights how phosphoinositide signaling is restricted to precise membrane compartments in order to maintain their identity," says Burd. PtdIns4*P* is generated at the TGN, where it recruits the effector protein Vps74 to spur the formation of retrograde transport vesicles. Vps74 and PtdIns4*P* are incorporated into these vesicles and delivered to earlier Golgi compartments, whereupon Vps74 recruits Sac1 to hydrolyze the phospholipid and terminate the signaling pathway.

Other membrane receptors may recruit Sac1 to different organelles to promote PtdIns4*P* turnover, and similar mechanisms may target other Sac1 family phosphatases. Mutations in the N-terminal subdomain of Sac3—corresponding to the region of Sac1 that interacts with Vps74—cause Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis (5, 6). "This provides a precedent for thinking about how these mutations cause disease," says Burd. "Sac3 might interact with membrane receptors that direct it to dephosphorylate its substrate phosphoinositide at specific organelles."

Burd and colleagues now want to further explore the importance of restricting PtdIns4*P* to the TGN and to investigate how Sac1 and Vps74 regulate sphingolipid homeostasis, given that mutations in either protein alter the sphingolipid content of Golgi membranes.

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