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SPOTLIGHT

Staying in touch: Taking a closer look at ER–Golgi contact sites

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ER–Golgi contact sites regulate lipid homeostasis and trafficking across the trans-Golgi network. However, their molecular nature is elusive. In this issue, Venditti et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201812020 and https://doi.org/10.1083/jcb.201812021) shine new light on the molecular determinants coupling lipid exchange and cargo exit with maintenance of ER–Golgi contacts.

The ER maintains close and dynamic contacts with virtually all other organelles through membrane contact sites (MCSs). MCSs are specialized subdomains of close membrane apposition (5–40 nm) operating as structural and molecular platforms for channeling materials and signaling molecules between organelles (1). One of the cardinal functions of MCSs, namely nonvesicular lipid transfer between organelles, was initially demonstrated by investigating subdomains of the TGN closely associated with the ER, called the ER–Golgi MCS (1). Mounting evidence indicates that the molecular machinery operating at the ER–Golgi interface is crucial to maintain the uneven and highly specialized lipid composition of the TGN, with high levels of sterol and phosphatidylinositol-4-phosphate (PI4P), which drives the formation of transport carriers and trafficking/sorting processes. Indeed, a lipid gradient across the ER–Golgi MCS is orchestrated by different families of lipid transfer proteins (LTPs), including oxysterol-binding proteins (OSBPs). LTPs typically contain a PH domain that binds to PI4P at the trans-Golgi surface, an FFAT (two phenylalanines [FF] in an acidic tract) motif interacting with the ER vesicle–associated membrane protein–associated proteins (VAPs), and a lipid transfer domain. This characteristic domain structure allows LTPs to operate both as a tether and lipid carrier. But how does this lipid transport machinery function? Are ER–Golgi MCSs essential for it? Recent studies have proposed a "countercurrent model" whereby OSBPs tethered at the ER–Golgi MCS transfer cholesterol from the ER to the trans-Golgi through a mechanism directly coupled to the countertransport of PI4P from the TGN, where it is produced (2). The removal of PI4P from the Golgi facilitates the hydrolysis of PI4P to phosphatidylinositol (PI) by the ER integral membrane PI4P-phosphatase Sac1, thus preventing the accumulation of PI4P at the ER. The back transfer of PI to the TGN allows PI4 kinases (PI4KIIIα and PI4KIIβ) to restore Golgi PI4P levels while maintaining OSBPs targeted at the MCS, thus closing the cycle. This energy-dependent PI4P turnover at the ER–Golgi interface is thought to drive the vectorial transport of sterol from the ER to the TGN, a cardinal component of the signaling and trafficking functions of the TGN (2). However, much remains to be learned about the essential components of the ER–Golgi MCSs providing the structural and spatial membrane environment regulating PI4P turnover. For example, while other OSBP-related homologues like ORP4, ORP9, and ORP10 have been described as lipid exchangers, their function and lipid ligand at the ER–Golgi MCS are poorly characterized. In this issue, Venditti et al. exploit the resolution power of a Förster resonance energy transfer–based approach to disentangle the structural molecular determinants of the lipid transfer machinery of the ER–TGN MCS, which they dubbed as ERTGoCS (3).

Through high-resolution EM combined with newly developed fluorescence lifetime imaging microscopy and fluorescence resonance energy transfer (FLIM–FRET), the authors imaged ERTGoCS in their native state. They found that 24% of the TGN surface is engaged in ERTGoCS, which are heterogeneous in terms of width (5–20-nm range) and location across the Golgi stacks (3). By monitoring the effects of the deletion or ablation of putative ERTGoCS candidates on FLIM–FRET signals, Venditti et al. show that, along with VAPs, ORP10 is indispensable for ERTGoCS stability, whereas ORP9 and OSBP1 have redundant roles and affect ERTGoCS only when their depletion is combined (3). Notably, the researchers found that ORP10 ferries phosphatidylserine (PS) from the ER to the TGN, thus operating as a PS/PI4P rather than as a cholesterol/PI4P exchanger, like OSBP1 and ORP9. Although the ability of ORP10 to bind and extract PS was reported in previous work (4), its relevance for ERTGoCS stability and PS redistribution through the TGN was unknown. Therefore, this finding expands the concept that MCSs are required functional coordinators of lipid countercurrents orchestrated by combined LTP activity and tethering functions. However, considering that depletion of ORP10 reduced the Golgi PS pool but did not affect the plasma membrane (PM) pool, it would seem that this two-lipid exchange is specific for the ER–Golgi lipid homeostasis but does not affect other pathways.

As predicted by the countercurrent model, disruption of ERTGoCS caused by the deletion of VAPs or ORP10 and the

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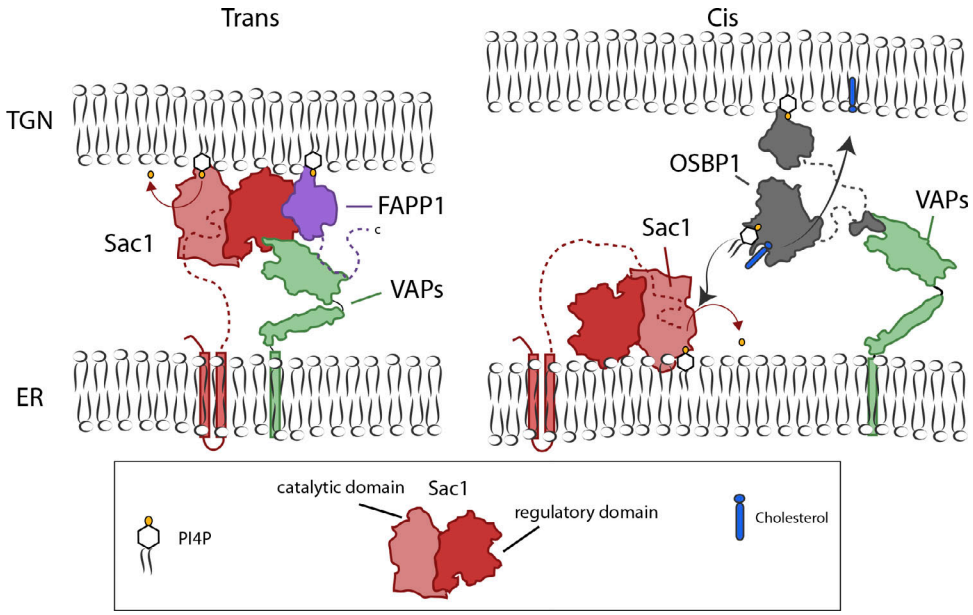

Figure 1. **The two hypothetical models suggested by the study of Venditti et al. (5), explaining the dual activity of Sac1 at the ER–TGN contact sites.** Tighter ER–TGN contact sites would be permissive of the in trans activity of Sac1, which, in the presence of the adaptor FAPP1, can access the TGN and hydrolyze PI4P. At broader contact sites, Sac1 would operate in cis mode and consume PI4P transported by OSBP1 from the TGN to the ER in exchange for cholesterol. Republished from Venditti et al. (5).

combined presence of ORP9 and OSBP1 resulted in the accumulation of PI4P at the TGN. A notable exception was the phosphatidyl-four-phosphate-adaptor-protein-1 (FAPP1), a still enigmatic TGN-associated protein that binds PI4P via its PH domain, whose absence increased Golgi PI4P levels without altering ERTGoCS (5).

Could FAPP1 directly affect the activity of a PI4P master regulator at the MCS? To answer this question, Venditti et al. used proximity biotinylation and in vitro binding assays with recombinant proteins (5). The researchers found that the molecular partners of FAPP1 are VAPs and Sac1. Superresolution microscopy and immuno-EM confirmed that FAPP1 localizes at the ERTGoCS and serves as an adaptor between PI4P-enriched TGN domains and the ER-associated PI4P phosphatase Sac1 (5). By reconstituting different configurations of enzyme–subtrate interactions in vitro, the authors further disclosed that FAPP1 markedly promotes the low in trans Sac1 phosphatase activity, even in the absence of VAP, but does not affect the more robust in cis Sac1 activity. To study the regulatory function of the FAPP1–Sac1 complex at ER–Golgi contacts in the cells, the researchers used an optogenetics-based approach or prompted ERTGoCS artificially by rapamycin-mediated FKBP-FRB heterodimerization. They found that, under conditions of forced ERTGoCS stabilization and depletion of VAPs, which abolished the anchoring of the cholesterol/PI4P exchangers OSBP1 and ORP9, the presence of FAPP1 was required to reduce the high PI4P levels in cells. Since under these conditions Sac1 activity can only occur in trans, the authors strengthened the point that FAPP1 modulates the in trans PI4P-degrading activity of Sac1.

How can this observation be reconciled with studies suggesting that Sac1 functions in cis at the ER–Golgi and ER–PM MCS to support lipid countertransport (2, 6–10)? To resolve the dual modes of Sac1 activity at the ERTGoCS, the authors predicted an interesting model whereby both in cis and in trans activities of Sac1 would coexist, depending on the ERTGoCS breadth (Fig. 1). At sites of closer contacts, the presence of FAPP1 would enable the in trans activity of Sac1 by positioning it adjacent to TGN domains of high PI4P concentrations, thus allowing its hydrolysis. Instead, at broader MCSs, Sac1 would hydrolyze PI4P transferred from the TGN to the ER by OSBP1 in cis. Although this prediction still needs to be validated by more experiments designed to modulate the distance of the ERTGoCS and their molecular composition in the cell, the fine-tuning of Sac1 activities, depending on the molecular architecture and the tethering partners of the MCS, may explain the controversial results reported in different studies (2, 6–8). However, it is possible that, at tighter MCSs, Sac1, which has a linker of up to 7 nm (11), functions in trans only when the adaptor FAPP1 is present at ERTGoCS. The biological significance of forming spatially and functionally heterogeneous subdomains regulating the in cis and in trans activities of Sac1 at ERTGoCS and other MCSs where it also localizes (1), such as the ER–PM and ER–endosome contacts, is still enigmatic.

But what is the overall functional meaning of these findings? Venditti et al. (5) revisited previous evidence that ORP10 interferes with the trafficking of the apoliproteinB100 (ApoB100), an essential component of the very low-density lipoprotein (VLDL) (12). In hepatocytes, VLDL is assembled in the ER, trafficked to the Golgi via specific ER-derived dedicated transport carriers, and secreted. An elevated secretion of hepatic VLDL is a feature of dyslipidemia, a condition triggering accelerated

artherosclerosis in metabolic diseases, like type 2 diabetes. Interestingly, mounting evidence involves OSBP/ORPs in dyslipidemia. By using pulse-chase analysis and synchronization assays to study ApoB100 trafficking across the TGN, Venditti et al. show that, in HepG2 cells, FAPP1, like ORP10, negatively controls the rate of ApoB100 export (5). Hence, FAPP1 functions both as a PI4P detector and Sac1 activator, able to regulate trafficking and secretion of specific cargoes, by fine-tuning the level of PI4P through the in trans activity of Sac1 across the ERTGoCS.

The exact mechanisms and modules linking key functions like sorting and trafficking across the Golgi stacks with PI4P gradients and dynamic ERTGoCS hetereogenity require further studies, but here, Venditti et al. shed new light on these fascinating questions.

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