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VIEWPOINT

Filling in the gaps: SNX-RGS proteins as multiorganelle tethers

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SNX-RGS proteins are molecular tethers localized to multiple interorganelle contact sites that exhibit roles in cellular metabolism. Here, we highlight recent findings on these proteins and discuss their emerging roles in metabolism, human disease, and lipid trafficking.

Making connections: SNX-RGS proteins as conserved interorganelle tethers

Pioneering electron microscopy in the 20th century by George Palade and colleagues revealed that eukaryotic cells are crowded landscapes filled with organelles. As might be expected in this dense environment, organelles physically contact one another, and such interorganelle contacts were noted by early cell biologists. It was not until decades later, however, and largely with the advent of technologies such as green fluorescent protein, that the protein machinery mediating these interorganelle junctions began to be deciphered. Over the past 20 yrs, there has been an explosion of research characterizing these "molecular tethers." They have emerged as key players not only in connecting organelles but also taking active roles in diverse cellular pathways. Beyond physical tethering, many function in metabolism, including nonvesicular lipid transport via lipid transport protein domains (LTPs) encoded within the tethers themselves.

One such protein tether family comprises the sorting nexin–regulator of G protein signaling (SNX-RGS) proteins, members of the larger SNX superfamily. Traditionally known as vesicle trafficking components, SNX proteins are generally soluble and contain Phox homology (PX) domains that bind specific phosphoinositide phospholipids (PIPs), enabling them to localize to distinct organelles decorated with their preferred PIP (Chandra et al., 2019). However, the SNX-RGS proteins are unique among the SNX superfamily in that they encode a conserved N-terminal transmembrane region that anchors them to the ER (Henne et al., 2015). True to their SNX nomenclature, SNX-RGS proteins also encode a C-terminal PX domain (PXC) that allows binding to other cellular compartments in trans (thus operationally defining SNX-RGS proteins as interorganelle "tethers"). Like other interorganelle tethers, SNX-RGS proteins are quite large (several contain >1,000 amino acids). Along this length are studded domains including the RGS domain (from which this SNX-RGS subfamily derives its name), as well as the poorly characterized PX-associated (PXA) and PXC domains unique to this protein family (Fig. 1, inset). Despite their high conservation, until recently the functions of SNX-RGS proteins were enigmatic compared with their more well-characterized SNX cousins. However, the past few years have witnessed a flurry of studies beginning to characterize these mysterious molecular tethers.

The purpose of this Viewpoint is to highlight recent findings on the SNX-RGS proteins and their roles in cell physiology and disease. Studies from several labs using yeast, *Drosophila*, and human cells reveal conserved roles for SNX-RGS proteins as organelle tethers and important regulators of lipid metabolism. Surprisingly, these studies also indicate a large degree of functional diversity among different SNX-RGS proteins. This functional diversification may be mediated by each homolog's unique preference for binding certain PIPs via its PX domain. Since organelles are decorated by specific PIPs that help create their identity, an emerging model is that differences in SNX-RGS PIP-binding preferences enable different SNX-RGS homologs to localize to distinct ER-organelle contacts throughout the cell (Fig. 1). A second emerging role for SNX-RGS proteins is their so-far universal ability to interact with lipid droplets (LDs), which bud from the ER network and serve as important lipid storage organelles. This LD interaction is distinct from contacts made with the ER and the PX-mediated organelle interaction, meaning that several SNX-RGS proteins are multiorganelle tethers localizing to so-called triorganelle junctions, the roles for which require additional study. In line with their attachment to multiple organelles, loss or mutation of specific SNX-RGS proteins perturbs organelle homeostasis and is associated with genetic diseases that provide clues to their functions. A final emerging highlight is that artificial intelligence algorithms such as Alphafold2 suggest that SNX-RGS proteins contain a putative LTP module that may be capable of nonvesicular interorganelle lipid transport. Surprisingly, this LTP module is created by the dimerization of two domains unique to SNX-RGS proteins, their PXA and

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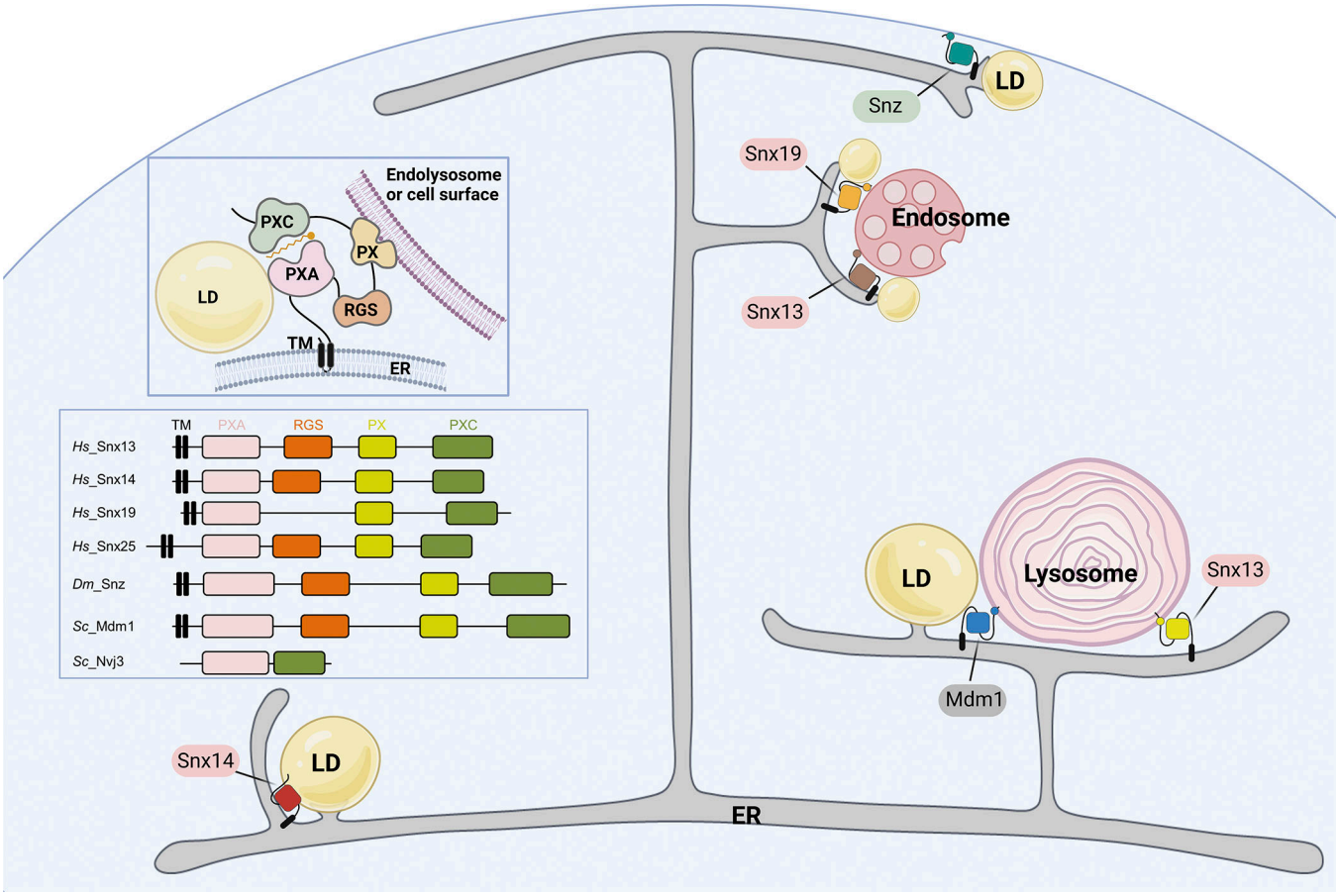

Figure 1. **SNX-RGS proteins are multiorganelle tethers.** Schematic of a subset of SNX-RGS proteins and their known subcellular localizations. Human Snx14 localizes to ER-LD contacts (Datta et al., 2019). Human Snx19 localizes to ER-endolysosome contacts (Saric et al., 2021). Human Snx13 localizes to ER-endosome/lysosome contacts (Lu et al., 2022). *Drosophila* Snz localizes to ER–plasma membrane contacts (Ugrankar et al., 2019). Yeast Mdm1 localizes to ER-lysosome (vacuole) contacts in budding yeast (also known as NVJs; Henne et al., 2015). Inset: A model SNX-RGS protein at a triorganelle junction composed of ER, LD, and an endomembrane compartment. The PX domain mediates contact with the endosome or plasma membrane. Here, the PXA and PXC domains are depicted forming a module with a large hydrophobic cavity, based on structural predictions (Paul, 2022). Inset also depicts multiple SNX-RGS orthologs in humans (Hs), *Drosophila* (Dm), and yeast (Sc).

PXC domains, which modeling suggests fold together into a module burying a large hydrophobic cavity capable (in principle) of housing lipids or metabolites. Given recent cell biological studies that imply a conserved role for SNX-RGS proteins in lipid binding or interorganelle transport, an emerging question is what role this LTP module has (if any) in lipid trafficking, and whether it could mediate nonvesicular interorganelle lipid exchange. This article will briefly cover these emerging issues and direct the reader to recent publications where they can learn more about the remarkable SNX-RGS proteins.

Here, there, everywhere: Spatial divergence of SNX-RGS proteins to different interorganelle junctions

With its well-developed genetic tools and ease of fluorescence imaging, the budding yeast *Saccharomyces cerevisiae* is an ideal model system to characterize interorganelle tethers. Yeast encode a SNX-RGS homolog termed Mdm1. Genetic and cell biological analysis indicate that the Mdm1 N-terminal transmembrane region is anchored to the ER network, while its C-terminal PX domain binds to phosphatidylinositol 3-phosphate (PI3P) enriched on the yeast vacuole (Henne et al., 2015). Mdm1 thus localizes to ER-vacuole contacts, and particularly in budding yeast to a unique interorganelle contact site known as the nucleus-vacuole junction (NVJ). The NVJ harbors several other interorganelle tethers and serves multiple purposes including a site for microautophagy (Roberts et al., 2003), sterol transport (Murley et al., 2015), and LD biogenesis (Hariri et al., 2018). Consistent with this last role, Mdm1 decorates LDs clustered at the NVJ, suggesting it labels ER-LD-vacuole triorganelle junctions (Hariri et al., 2018). Further dissection revealed that Mdm1 acts as an adaptor for recruiting the fatty acyl-CoA ligase Faa1 to the NVJ, where it promotes local fatty acid activation for incorporation into triglycerides housed within LDs (Hariri et al., 2019).

A clear homolog to Mdm1 is human Snx19, which shares the Mdm1 domain architecture. Recent work reveals that, like yeast Mdm1, Snx19 is anchored to the ER network of U-2OS cells, while its C-terminal PX domain binds PI3P on endolysosome compartments (Saric et al., 2021). As expected of a tether, SNX19 ablation reduces ER-endolysosome contacts and causes LAMP1-positive lysosomes to disperse and accumulate in the cell periphery, suggesting that Snx19 may influence lysosome spatial

positioning. Snx19 was also recruited to the surfaces of LDs following stimulation with oleate, a potent LD biogenesis fatty acid. Collectively, these data suggest that human Snx19 exhibits triorganelle localization similar to that of yeast Mdm1, and may influence organelle positioning.

Humans encode four SNX-RGS proteins: Snx13, Snx14, Snx19, and Snx25. Surprisingly, not all these Mdm1 orthologs localize to ER–endolysosome contacts. Like its homologs, Snx14 also localizes to the ER network via its N-terminal transmembrane region. However, two independent studies indicate it does not interact with endolysosome compartments (Datta et al., 2019; Saric et al., 2021). This is attributed to its "broken" PX domain, which is naturally mutated to not bind strongly to PIPs (Mas et al., 2014). Like all examined SNX-RGS proteins, Snx14 interacts with LDs and enriches at ER-LD contacts following the stimulation of LD biogenesis with oleate in U-2OS cells. In line with this, Snx14-knockout cells display defective LD morphology, as well as lipotoxic sensitivity when exposed to saturated fatty acids (Datta et al., 2019, 2020).

A second example of SNX-RGS proteins localizing to spots other than ER–endolysosome contacts occurs in *Drosophila melanogaster*. *Drosophila* encode only one SNX-RGS protein, termed Snazarus (Snz), initially linked to organismal aging (Suh et al., 2008). Snz is highly expressed in the *Drosophila* adipose tissue called the fat body, where it localizes to ER–plasma membrane contacts at the tissue surface (Ugrankar et al., 2019). This localization is attributed to its PX domain, which primarily binds to phosphatidylinositol 4,5-bisphosphate at the cell surface, and not to PI3P like other SNX-RGS proteins (Mas et al., 2014; Ugrankar et al., 2019). Snz also interacts with LDs in the fat body, and its deletion alters LD morphology. Notably, fat body–specific Snz overexpression increases fat storage and *Drosophila* starvation resistance, indicating a role for Snz in energy storage within adipose tissues. The closest homolog to Snz in humans is Snx25, which also associates with LDs following oleate addition and appears to play an additional role in autophagic flux, but its full function remains to be determined (Lauzier et al., 2022; Paul, 2022). Although further study is required, these initial studies indicate that different SNX-RGS proteins localize to distinct interorganelle contacts but may universally interact with LDs, particularly during stimulated LD biogenesis.

Triorganelle contacts in the lipid metabolism nexus

Given their ability to bind to LDs, the ER network, and a tertiary organelle such as endolysosomes or the cell surface, what is the functional importance of SNX-RGS proteins at triorganelle contacts? One possible role is the shuffling of lipids between these organelles. Other proteins are now observed to localize at similar three-way organelle junctions, such as MIGA2 in adipocytes, where they play such a role (Freyre et al., 2019). MIGA2 is thought to promote de novo triglyceride synthesis at ER-LD-mitochondria triorganelle junctions from lipids derived from carbohydrates in the adipose tissue. These triglycerides are stored in LDs and available for harvesting in nearby mitochondria via fatty acid oxidation, painting MIGA2 as an energy homeostasis regulator that connects organelles involved in the synthesis, storage, and mobilization of lipids. Similarly, SNX-RGS proteins may link the lipid synthesis (ER) and storage (LDs) organelles to organelles that are sources of lipid precursors. For example, *Drosophila* Snz may help scavenge lipids at the plasma membrane that are derived from the extracellular insect hemolymph, enabling them to be delivered to the cortical ER where they are incorporated into triglycerides and stored in peripheral LDs. Indeed, Snz overexpression enhances adipose fat storage and also the incorporation of dietary lipids such as α-linoleic acid into triglyceride, which originates from the circulating hemolymph (Ugrankar et al., 2019).

Getting to the fat of the matter: SNX-RGS proteins in lipid biology and disease

With their conserved role localizing to multiorganelle contacts, what then is the general function of SNX-RGS proteins, and how does this contribute to cell homeostasis? Several findings indicate a role in pooling or transporting lipids between organelles. Using unbiased CRISPR-based screening, recent work suggests a role for Snx13 in regulating cholesterol export from lysosomes (Lu et al., 2022). Normally, exogenous cholesterol is delivered to cells through the endocytosis of lipoprotein particles and their trafficking to lysosomes, where cholesterol is exported via NPC1. Cells with dysfunctional NPC1 accumulate lysosomal cholesterol. Surprisingly, loss of Snx13 reduced lysosome cholesterol accumulation in NPC1-defective cells, suggesting that Snx13 is a potent negative regulator of lysosome cholesterol efflux (Lu et al., 2022). Snx13-deficient cells also accumulated the mysterious lipid bis(monoacylglycero)phosphase (BMP) in their lysosomes. Given that BMP itself can regulate cholesterol efflux, a tempting hypothesis is that Snx13 may influence BMP pools or interorganelle trafficking. Snx13-deficient cells also accumulated LDs, and labeled Snx13 was visualized in close proximity to LDs as well as the ER and lysosomes, underscoring its role in multiorganelle cross talk.

There is some evidence that SNX-RGS proteins play important roles in development, and their loss contributes to human disease. Genetic perturbation of Snx14 results in a homozygous recessive cerebellar ataxia disease called SCAR20. Clinical features of SCAR20 include delayed psychomotor development and cerebellar atrophy (Thomas et al., 2014; Akizu et al., 2015). Snx14-deficient cells exhibit perturbed LD morphology, altered neutral lipid metabolism, and enhanced sensitivity to fatty acid–induced cell death, all suggestive of defective lipid homeostasis (Bryant et al., 2018; Datta et al., 2020). Similarly, Mdm1-deficient yeast are sensitive to fatty acid lipotoxicity (Hariri et al., 2019). Snx13- and Snx14-knockout mice have been created, and both manifest embryonic lethality, indicating an essential and likely nonredundant role for mammalian SNX-RGS proteins in murine development (Zheng et al., 2006; Bryant et al., 2020). Collectively, these findings suggest that SNX-RGS proteins maintain cellular lipid homeostasis, and their loss alters cellular lipid profiles and sensitizes cells to lipotoxicity.

Following the lipid flow: SNX-RGS proteins as putative LTPs

A final recent noteworthy finding suggests a role for SNX-RGS proteins in nonvesicular interorganelle lipid transport. Breakthroughs in artificial intelligence algorithms such as Alphafold2 enable the accurate prediction of protein folding. Strikingly, the predicted structures of several SNX-RGS

proteins suggest that their PXA and PXC domains fold together into a module containing a large hydrophobic cavity capable of enclosing lipids (Paul, 2022). Although only an in silico prediction, this implies that SNX-RGS proteins are capable of binding and potentially transporting lipids between membranes via this LTP-like module, in a manner reminiscent of other LTP domain–containing tethers such as the SMP domain–containing extended-synaptotagmin proteins (Schauder et al., 2014; Fig. 1, inset). Further experimental studies are necessary to reveal whether this module truly exists in SNX-RGS proteins, and what lipids or other biomolecules it may bind.

Closing the gaps: Deciphering the molecular functions of SNX-RGS proteins

Despite these new findings, the precise molecular functions of SNX-RGS proteins remain unclear. More study is needed to decipher precisely how each SNX-RGS protein at its respective interorganelle junction influences cell and lipid homeostasis. Because all SNX-RGS proteins examined to date interact with LDs, the precise role for these factors in LD biology also needs further investigation. As loss of SNX-RGS proteins generally alters LD morphology and sensitizes cells to lipotoxicity, a tempting model is that SNX-RGS proteins influence the exchange of lipids or fatty acids between LDs and other organelles. However, human SNX-RGS proteins only visibly decorate LDs after oleate addition, implying that their LD interactions are not absolute but rather are dependent on metabolic context. A third and pressing focus should be on testing the hypothesis that SNX-RGS proteins encode LTP-like modules capable of nonvesicular lipid exchange between closely opposed membranes. Additionally, understanding the role of the RGS domain, which may influence GTP hydrolysis of G-α subunits associated with GPCR signaling, is an unresolved aspect of these proteins. No doubt there is still much to learn about these fascinating multi-organelle tethers and their roles in health and disease.

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