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SPOTLIGHT

Spastin joins LDs and peroxisomes in the interorganelle contact ballet

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Lipid droplets (LDs) store energy-rich fatty acids (FAs), but to harvest them, LDs donate FAs to peroxisomes for oxidation. In this issue, Chang et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201902061) identify M1 Spastin as an LD–peroxisome tether that interacts with ESCRT-III components to facilitate lipid exchange between these two organelles.

Life presents energetic challenges, and cells have developed ways to store excess energy so they can subsist during periods of nutrient shortage. The major eukaryotic nutrient storage organelle is the lipid droplet (LD), a unique cytoplasmic compartment composed of an outer phospholipid monolayer and filled with energy-rich fatty acids (FAs) in the form of triacylglycerides (TAGs; 1). LDs store FAs, but to be harvested for energy they must be imported into oxidative organelles like mitochondria or peroxisomes, where they are metabolized through β-oxidation. This interorganelle FA trafficking presents a significant challenge for the cell; FAs act as detergents that dissolve biological membranes and cause lipotoxicity. The cell has developed an elegant solution to the FA-trafficking problem: LDs form intimate contact with oxidative organelles, and these interorganelle junctions serve as conduits for FA flux, thus avoiding the need to spill FAs into the cytoplasm, where they would cause havoc.

Contact sites formed between LDs and peroxisomes have been observed for decades by electron microscopy, but the molecular machinery that connects these organelles together has remained largely unclear (2). In this issue, new work from Chang et al. sheds some light on LD–peroxisome tethering by demonstrating that the AAA ATPase M1 Spastin can directly regulate LD–peroxisome contact and promote FA interorganelle exchange (3).

The canonical role for Spastin is in microtubule severing, but Spastin exists as two major isoforms, with the longer M1 variant encoding an N-terminal hydrophobic hairpin motif that targets it to LDs (4). Spastin is also one of many proteins implicated in hereditary spastic paraplegias (HSPs), a growing family of genetic diseases associated with neurological pathology and lower-extremity spasticity (5). Intriguingly, recent work reveals that many HSP-associated proteins have roles in FA metabolism or LD homeostasis. Indeed, Spastin-deficient *Drosophila melanogaster* and *Caenorhabditis elegans* exhibit defects in fat storage, and cells from HSP patients with Spastin mutations show defects in peroxisome homeostasis, suggesting Spastin plays some role in lipid homeostasis (4). Chang et al. not only show that M1 Spastin targets to LDs, but that its overexpression promotes LD–peroxisome contacts (3). Conversely, knockdown of endogenous M1 Spastin in metabolically stressed cells reduces LD–peroxisome contacts, indicating a role for Spastin in LD–peroxisome tethering.

If Spastin acts as a tether, how does it connect LDs to peroxisomes? Chang et al. demonstrate that this tethering requires the N-terminal hydrophobic hairpin of Spastin, suggesting that direct insertion into the LD monolayer surface is crucial for tethering (3). They also characterize a peroxisome-interacting (PXI) region in the middle of Spastin that is required for peroxisome recruitment to LDs. This PXI region is sufficient to coimmunoprecipitate with ABCD1, a peroxisome surface protein and long chain FA importer (Fig. 1). In line with this, knockdown of ABCD1 suppresses Spastin-induced LD–peroxisome tethering, suggesting that M1 Spastin forms an interorganelle bridge together with ABCD1. Whether this Spastin–ABCD1 coupling directly facilitates FA channeling into peroxisomes remains unclear.

To enter peroxisomes, FAs must first be removed from the LD core. How this extraction is coordinated remains enigmatic, but Chang et al. make the surprising observation that components of the endosomal sorting complexes required for transport (ESCRT) III machinery contribute to this interorganelle FA flux (3). ESCRT-III is composed of numerous soluble proteins that polymerize into hetero-oligomeric rings and spirals to deform membranes and drive organelle remodeling (6). Due to their unique membrane-sculpting properties, nature has incorporated ESCRT-III assemblies into diverse cellular functions, including multivesicular body biogenesis, viral budding, cytokinesis, and nuclear envelope remodeling to name a few. Loss of ESCRT-III subunits has also been observed to perturb cellular lipid homeostasis, but whether this was a direct effect or indirect through general defects in endomembrane trafficking was unclear. Here, Chang et al. find that M1 Spastin overexpression can recruit ESCRT-III subunits IST1 and CHMP1B to the surfaces of LDs (Fig. 1). This recruitment is dependent on the Spastin MIT domain, which interacts with MIM motifs present on ESCRT-III subunits (7). Surprisingly, mutant Spastin unable to recruit IST1/CHMP1B causes defects in

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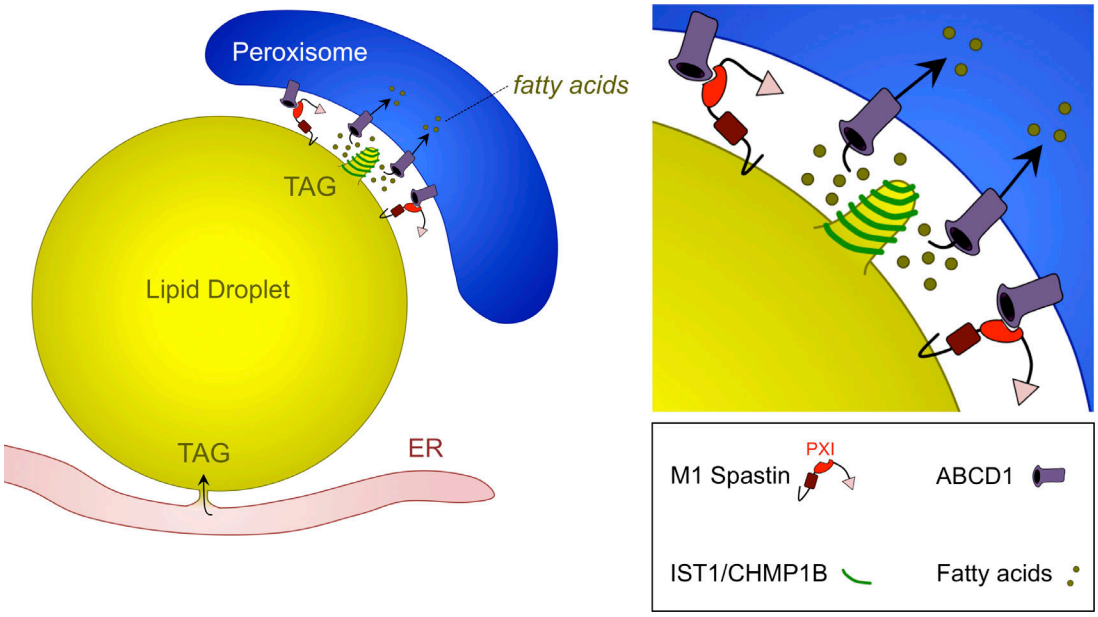

Figure 1. **M1 Spastin tethers LDs to peroxisomes and promotes FA exchange.** M1 Spastin interacts with peroxisome FA importer ABCD1 to connect LDs to peroxisomes. Spastin interacts with ABCD1 via its PXI region. Spastin can also interact with ESCRT-III subunits IST1 and CHMP1B, which are capable of polymerizing into membrane-bending hetero-oligomers.

fluorescent FA exchange between LDs and peroxisomes, even though this Spastin mutant is sufficient to tether these organelles together. This implies that ESCRT-III subunits may play active roles in lipid exchange at the LD–peroxisome interface, potentially by implementing their membrane-bending capacity to promote lipid extraction out of LDs. In line with this, many ESCRT-III proteins contain lipid bilayer–inserting amphipathic helices that would be capable of penetrating the LD monolayer surface and promoting lipid extraction (8). IST1 and CHMP1B also assemble into cone-like polymers that could promote LD surface remodeling and potentially promote lipid exchange (9). However, many key questions remain. How this ESCRT-III–mediated LD remodeling would interface with the lipolysis necessary to convert TAGs into soluble FAs before peroxisome import remains unresolved. Also, ESCRT-III complexes typically associate with membrane bilayers, so how they would interact with monolayers is unclear, although ESCRT-III spirals and rings can assemble on supported lipid monolayers in cell-free assays (10).

In addition to harvesting FAs for energy production, peroxisomes help maintain cellular homeostasis by recycling lipids and helping protect cells from oxidative stress and damage. Using a tissue culture–based system, Chang et al. demonstrate that Spastin overexpression can reduce peroxidated lipid accumulation in cells exposed to oxidative stress (3). Furthermore, this protective effect appears dependent on Spastin's LD–peroxisome tethering ability, as expression of an autosomal dominant HSP patient-derived mutant, Spastin^{K388R}, was unable to promote LD–peroxisome tethering and also failed to lower peroxidated lipid levels. This intriguing observation suggests that Spastin-mediated LD–peroxisome tethering may play important roles in cellular lipid homeostasis, the loss of which could contribute to the neuropathology observed in HSP patients.

In closing, LD–peroxisome contacts are evolutionarily conserved interorganelle junctions used by yeasts and mammals to maintain energy homeostasis and protect against lipotoxicity. Identifying M1 Spastin as an LD–peroxisome tether that influences FA exchange provides new insights into the mechanisms governing interorganelle crosstalk and furthers our understanding of HSPs and related diseases. It also expands our knowledge of Spastin-interacting proteins like ABCD1 and IST1/CHMP1B, which may play unexpected but important roles in lipid trafficking and homeostasis. However, many questions still remain. Recent studies reveal that both LDs and peroxisomes can be created by sprouting from the same subdomain of the ER (11, 12). This indicates that LD–peroxisome crosstalk may occur throughout the life of these organelles and through their connections with the ER. How this tri-organelle crosstalk is regulated, and how it interplays with M1 Spastin-induced LD–peroxisome tethering remains unclear, but it is central to our understanding of FA metabolism and interorganelle lipid flux. Furthermore, how cells sense lipid peroxidation and mount an orchestrated multi-organelle response to maintain lipid homeostasis remains poorly understood, but is likely critical to our understanding of many diseases. Indeed, new work continues to reveal disease-related proteins like Rab18, Snx14, and Mysterin that play unexpected but important roles in lipid metabolism and LD crosstalk with other organelles (13–15), but how these proteins work together to connect LDs with other cellular compartments requires further study.

Once thought to be an inert storage compartment, the gregarious LD is quickly becoming recognized as the life of the interorganelle communication party, with LD interorganelle crosstalk playing important roles in energy and lipid homeostasis. No

doubt the dandy LD and its dancing partner the peroxisome will continue to intrigue us by the contacts they form, and by how these in turn influence cellular and organismal lipid homeostasis.

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