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SPOTLIGHT

Building the lipid droplet assembly complex

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In this issue, Choudhary et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201910177) address the nature of the ER subdomain from which lipid droplets emanate and how several assembly proteins interact. Their data indicate that seipin/Nem1 marks these sites and provide a detailed working model for assembling the protein complex.

As we learn more about the multiple roles of cytoplasmic lipid droplets (LDs) in energy homeostasis, protection from lipotoxicity, storage of bioactive lipids such as retinal esters and proteins such as histones, interorganellar communication, and as a platform for the assembly of pathogens (1), interest has grown about the formation of these bona fide organelles. LDs are assembled from neutral lipids originating in the ER. Monomers of triacylglycerols (TGs) and steryl esters (SE; and others in smaller amounts) coalesce in the bilayer, and these aggregates initially are very mobile in the plane of the membrane (2). The neutral lipid cores become stabilized and grow, budding out toward the cytoplasm.

No particular assembly protein is required for LD formation. LDs can readily form from synthetic lipid bilayers after they incorporate a rather small but destabilizing amount of neutral lipid. However, several proteins are normally important for organizing and regulating droplet formation in cells. In their absence, droplets are radically heterogeneous in size, can readily aggregate and fuse (rare in nature), inappropriately break off from the ER membrane, and do not contain the normal subset of proteins. Assembly factors are therefore clearly important for LD stability and function, and communication with other organelles. We have learned much recently about the function of several of these factors, including seipin, FIT2 family proteins, the lipin complex, and Pex30 (and there are several others), but the bulk of work has focused on the function of individual factors.

The paper by Choudhary et al. in this issue (3) addresses two questions: First, do lipid droplets bud from random locations in the ER; where do the mobile neutral lipid aggregates settle down? Second, how do the known factors collaborate to establish the machinery for droplet formation?

There is already evidence that lipid droplets do not bud anywhere in the ER. A report several years ago demonstrated that droplets emanate from ACSL3 sites in cells (4). Past work has indicated that LD budding favors ER tubules rather than sheets, most recently in compelling and elegant experiments by Santinho et al., demonstrating in several ways that triacyglycerols are more unstable and energetic in tubules, ready to bud out into LDs (5). Importantly, this work also showed that the LD assembly factor seipin also resides mainly in ER tubules. It is there to control budding in a way that allows the association of relevant proteins onto the droplet, preventing ectopic and chaotic LD formation.

The Choudhary et al. paper complements this story nicely by showing that much of seipin is immobile in the yeast membrane, even without neutral lipids in the cell, shown with a yeast mutant strain that cannot synthesize TG or SE. Subsequent data support the conclusion that it is these immobile sites in the ER (presumably at tubules or edges of sheets) that will birth LDs. The issue, as yet unresolved, is the nature of the forces and molecules—perhaps cytoskeleton, traffic congestion from other immobile ER elements, or a patch of specialized lipid—that keep seipin immobilized in the membrane independent of neutral lipids.

While the immobility of seipin is interesting and important, the major strength of the Choudhary et al. paper is elucidating the order in which other LD assembly factors are assembled. The system employed is the induction of a known TG synthase, Lro1, in a strain background in which the other neutral lipid synthetic enzymes are all knocked out. Lro1, a transmembrane protein, has its active site facing the ER lumen. In a special twist, the authors express the luminal domain of Lro1 (sLro1) fused with a phenylalanine-phenylalanine-acidic tract (FFAT) sequence that is known to bind to the ER protein Scs2. This construct produces TG efficiently once induced even though the active site of the enzyme now faces the cytosol. They then observe, through fluorescence microscopy, the dependence of binding of Lro1 or other proteins to seipin and other LD factors.

First up is Nem1, a member of a phosphatase complex that activates the DG-synthetic protein Pah1 (lipin in animals). Nem1, with its partner Spo7, forms puncta in cells adjacent to LDs, similar to seipin, and the group now demonstrates that these puncta exist even without neutral lipids, and they are exclusively immobile. Once Lro1 is induced, it quickly binds to Nem1 and

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Fld1 (seipin, also termed Sei1) puncta. (A subset of Nem1 puncta localize with Fld1 even without neutral lipids, and this overlap increases as Lro1 is induced.) This is comforting, as the generation of TG by Lro1 is spatially linked to its storage site. Surprisingly, the behavior is even stronger with the FFAT-sLro1 than with the native Lro1. Importantly, both Nem1 and Fld1 are necessary for Lro1 puncta formation. There are two fascinating parts to this story: First, a catalytically dead Lro1 does not bind to the complex, suggesting that TG is acting as an intermediary in binding. Second, the role of Nem1 can be replaced by a constitutively acting Pah1, indicating that DG is an important element in assembly of the complex, and this is borne out with a DG probe, which is shown to localize the Nem1/Fld1 complex. It is unclear how or whether DG, the product of Pah1, finds its way to the complex in the absence of Nem1; is it binding to the Nem1 partner Spo7?

Next the authors examine Yft2, a FIT2 homologue that is important for ensuring cytosol-directed LD budding, as previously shown by authors in the group (6). Yft2 can join the complex even in the absence of seipin as long as Nem1 is present. Again, DG is likely involved in this interaction as the constitutively active Pah1 can substitute for Nem1.

Pex30, a reticulon-like protein, was originally found to be important for peroxisomal formation and more recently shown to interact with seipin, connecting LD and peroxisomal formation (7, 8). Choudhary and colleagues now show that Pex30 is absolutely essential for binding of Lro1, although it is not required for Yft2 binding. Whether Yft2 binds before Pex30 or independently is not clear.

The yeast perilipin Pet10 (Pln1), important for LD stability and for the regulation of protein trafficking to droplets (9), is not required for any of the former steps, suggesting either that it binds onto droplets at the end of assembly (the authors' preference), or that binding is independent of the other factors and can occur once it senses TG aggregates from the cytosol.

Based on all these observations, the authors have hypothesized an eight-step process of LD assembly. To my knowledge, this is the most detailed schema thus far and should serve as a facile blueprint for confirmation and extrapolation. And there are important details in this report that require investigation. As mentioned earlier, we do not know how seipin and Nem1 are anchored in the ER and how constitutively active Pah1 is able to overcome Nem1 in the complex. It is not clear, and quite surprising, that the Lro1 can interact with the complex from either side of the ER membrane, although this could be through TG. We do not know how neutral lipid aggregates are captured by Nem1/seipin, although the seipin-binding protein LDAF1 is a good candidate (10). We do not know which of the protein–protein associations described here—the stoichiometries of which are totally unknown—are direct versus indirect, or whether lipids such as DG or TG are required. Assuming neutral lipid aggregates encounter seipin/Nem1 at highly curved ER membranes, we do not know what further role membrane geometry has in the other associations although it is likely to attract Pex30. But even with all these outstanding questions, a detailed model of the assembly machinery for generating LDs has now been offered with ample support. Further developments based on this model are eagerly anticipated.

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