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SPOTLIGHT

ORP5 regulates PI(4)P on the lipid droplet: Novel players on the monolayer

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How the distinct lipid composition of organelles is determined and maintained is still poorly understood. In this issue, Du et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201905162) show that the lipid transfer protein ORP5 functions at ER–LD contact sites, regulating lipid droplet levels of phosphatidylserine and phosphatidylinositol-4-phosphate.

Lipid droplets (LDs) are highly dynamic organelles that are found in virtually all cells and are involved in a plethora of cellular functions (1). LDs play a pivotal role in lipid and energy homeostasis. Functioning as a storage depot of neutral lipids (NLs), LDs can sequester excess fatty acids mainly in the form of triacylglycerol (TAG) and steryl esters. The number and size of LDs vary depending on the metabolic state, increasing under conditions of nutrient profusion and decreasing when nutrients are scarce. When needed, stored NLs can be hydrolysed, and the fatty acids released can be used as building blocks for membrane biogenesis, or for the production of energy by β-oxidation.

LDs are formed in the ER, originating from a lens of NLs that coalesce. This "nascent LD" grows and buds to the cytosolic face of the ER, where the cytosolic leaflet of the ER coats the NL core, yielding the unique LD monolayer surface. Budded LDs can expand further by growth or fusion of smaller LDs. Upon growth of LDs, the monolayer surface must expand in order to reduce the surface tension on the LD. However, where the phospholipids required for LD expansion originate from remains a key question. LDs have also been observed to "dock" onto the ER, with the possibility of forming a lipid "stalk" or "bridge." This membrane connection between the ER and LDs could provide a means of lipid transport, facilitating lipid diffusion through the lipidic bridge. Alternatively, phospholipids could be delivered to LDs by specialized lipid transfer proteins, many of which are localized at organelle contact sites (2).

ORP5 is a member of the oxysterol binding protein (OSBP)–related protein (ORP) superfamily, which are lipid transport proteins (LTPs) that can bind sterols, phosphatidylserine (PS), and phosphoinositides (PIPs; 2). Previously, ORP5 has been proposed to mediate the exchange of PS and phosphatidylinositol-4-phosphate (PI(4)P) between the ER and plasma membrane (PM; 3). In this issue, Du et al. demonstrate that ORP5 also functions as an LTP at ER–LD contact sites (Fig. 1). Interestingly, whereas the targeting of ORP5 to ER–PM contacts previously was shown to depend on the pleckstrin homology (PH) domain (2), localization of ORP5 to LDs was shown to be dependent on its OSBP-related domain (ORD; 4). Moreover, an apparent amphipatic helix in the ORD was proposed to mediate the LD targeting.

In cells devoid of ORP5, LDs are larger and cellular TAG levels are increased; thus, ORP5 activity likely mediates LD size. Using fluorescent lipid sensors, Du et al. show that loss of ORP5 decreases the PS content of LDs and increases PI(4)P, indicating that ORP5 exchanges PI(4)P from the LD with PS from the ER (4; Fig. 1). Finally, they demonstrate that the PI(4)P on LDs is synthesized by PI 4-kinase 2-α (PI4K2A; Fig. 1).

The inositol headgroup of PI possesses three hydroxyl groups that are accessible for phosphorylation (D3, D4, and D5 hydroxyls). Phosphorylation of a single or multiple hydroxyl sites yields seven possible PIPs in mammalian cells, providing a signaling platform at the membrane surface with eight options (including unphosphorylated PI). PI(4)P and PI 4,5-bisphosphate (PI(4,5)P_2) are the most abundant PIPs in mammals and are conserved from yeast. Although of low abundance, PIPs play an essential function as signaling molecules, mediating the regulation of various cellular processes, including metabolism and energy homeostasis (5). LDs are a major axis of energy storage and homeostasis, and PIP signaling has previously been indicated to be involved in maintaining LD homeostasis (6). However, besides the implication of the minor PIP, PI 5-monophosphate (PI(5)P), being present on LDs (7), PIP signaling on LDs remains largely undescribed. In this study, Du et al. show for the first time that PI(4)P is present on LDs (4).

At the moment, one could only speculate on the possible roles for PIPs on LDs. PI(4)P concentration gradients are used to establish directionality on ORP-mediated lipid transport (2), as is likely the case for ORP5 transporting PS to LDs. It is possible that other ORPs use LD PI(4)P to transport other lipids, such as sterols, to LDs. Evidence is accumulating that cells host different subpopulations of LDs, varying in size, metabolic activity, and contact sites, with other organelles (8). The "labelling" of the LD monolayer with different PIPs could provide a way to facilitate recruitment of proteins to specific LD populations. In addition, PIPs have been implicated in mediating formation of organelle contact sites, e.g., PI(4)P mediating ER–PM contacts; therefore, it is possible that LD PIPs regulate specific LD contact sites. Under stress conditions, LD behavior changes, having an altered cellular localization and organelle contacts (9). Under

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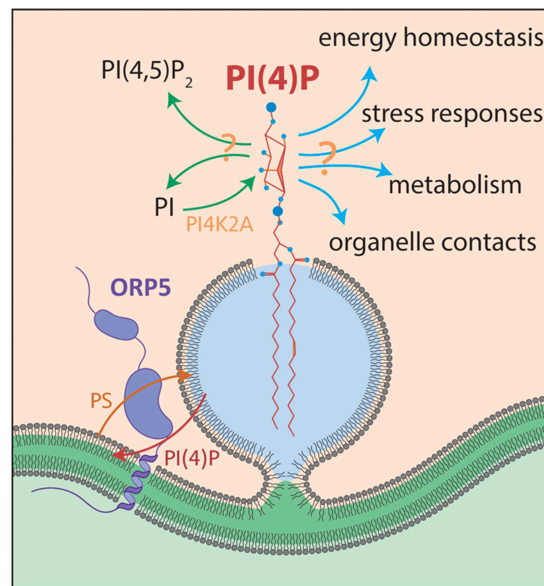

Figure 1. **ORP5 mediates LD PI(4)P levels by exchanging LD PI(4)P with ER PS.** PI(4)P on LDs is synthesized from PI by PI4K2A and may be phosphorylated to PI(4,5)P_2 or dephosphorylated to PI. LD PI(4)P could function as a signaling molecule in various signal transduction pathways, including energy homeostasis, stress responses, metabolism, and regulation of LD contact sites.

nutrient deprivation, a subset of LDs is recruited to the vacuole/lysosome, forming organelle contacts, followed by turnover by lipophagy. As PIPs can initiate autophagy (10), it is possible that PIP signaling plays a role in the regulation of lipophagy.

The identification of PI(4)P on the LD by Du et al. not only brings many new and interesting questions to the field of LD biology, but also implies an important role(s) of PIPs in governing inter-organelle crosstalk to coordinate numerous cellular responses. Although technically challenging, isolation of different LD populations from cells under various conditions and analysis of their full lipidome, including PIPs, will provide insights to whether—and how—PIPs are used for signal transduction purposes in LD homeostasis.

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