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Lipid droplet biogenesis in *Saccharomyces cerevisiae*

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Lipid droplets (LDs) are globular cellular organelles that store energy in the form of neutral lipids, particularly triacylglycerol (TAG) and sterol ester (STE). They are distinguished by being enclosed by a phospholipid monolayer harboring lipid biosynthetic enzymes and structural proteins such as perilipins (PLINs). LDs emerge from the endoplasmic reticulum (ER) where the acyltransferases that synthesize TAG and STE reside. This observation supports a model of LD biogenesis in which neutral lipids accumulate between the two leaflets of the ER membrane as oil lenses, which grow in size and mature in their protein composition to yield LDs. Eventually, LDs may bud to the cytosol or stay connected to the ER.

We probe the topological relationship between LDs and the ER by targeting the mammalian lipid droplet markers, perilipins (PLINs) into the lumen of the ER. Therefore, PLINs were fused to an ER signal sequence and their subcellular localization was analyzed. Remarkably, in wild-type cells, the ER luminal probes are capable of localizing to nascent and mature LDs, indicating that LDs are accessible to ER luminal proteins, and thus not independent cytosolic structures as frequently stated. Next, we tested whether ER integral membrane protein can localize to the LD surface. We expressed ER integral membrane protein fused to the mammalian PLIN3 in yeast and mammals. The ER integral membrane proteins that function in a bilayer can associate with the LD surface in both yeast and mammals. These data indicate that the LD surface is not only accessible to cytosolic amphipathic proteins or ER hairpin containing proteins, but also to bilayer-spanning integral membrane proteins, suggesting that the LD surface is continuous with the ER membrane.

Jury:
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