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SPOTLIGHT

Lipid droplets go through a (liquid crystalline) phase

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Lipid droplets (LDs) are key organelles for fat storage and trafficking. In this issue, Rogers et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202205053) show that glucose restriction triggers liquid crystalline lattice formation within LDs, which in turn alters the recruitment of proteins to the LD surface.

Lipid droplets (LDs) are key organelles that store neutral lipids. They have a unique structure consisting of a phospholipid monolayer surrounding a hydrophobic core of triglycerides (TGs) and sterol esters (SEs; 1, 2). Previously, cryo-electron tomography (cryo-ET) revealed that under certain conditions, striking onion-like layers appear within the neutral lipid core of LDs (3). However, the function of this organization was unknown. In this issue, Rogers et al. (4) show that glucose restriction triggers LD cores to form liquid crystalline lattices (LCLs). Importantly, they show that liquid crystalline phases are associated with selective remodeling of the LD proteome. Thus, this work provides insight into the mysterious function of a previously observed structure, while also elucidating a novel mechanism whereby LD proteome composition and heterogeneity are regulated.

Cholesterol ester–rich LDs are enriched in specific tissues in the body and accumulate in diseases such as atherosclerosis and non-alcoholic steatohepatitis (5, 6). In vitro studies going back decades have demonstrated that unsaturated cholesterol esters can form a liquid crystalline mesophase at physiological temperatures (7). This liquid crystal phase has a "smectic" organization, in which molecules are arranged into layers one molecule thick with their long axes perpendicular to the layers (Fig. 1). Smectic liquid crystals exhibit birefringence when illuminated with polarized light, as do LDs in cells loaded with cholesterol (7, 8). Previous work using cryo-ET demonstrated that LDs can exist in a smectic liquid crystalline phase in mitotically arrested or starved HeLa cells, exhibiting a pattern of concentric layers (3). These LCL-LDs exhibited tight membrane contact sites with mitochondria, suggesting that they are functionally different from other LDs. However, the physiological cues that regulate the phase state of LDs, and how the protein composition and function of LCL-LDs differs from other LDs, was unclear.

These studies by Rogers and colleagues (4) build upon previous work that characterized the upregulation of sterol precursor synthesis in response to acute glucose restriction (AGR) in yeast (9). Using cryo-ET, the authors examined if AGR changed the structure of LDs. Remarkably, LDs under AGR exhibited a striking morphological shift. The neutral lipid core of the LDs, which under standard growth conditions appears uniform, now consisted of a series of periodic concentric rings. Yeast LCL-LD formation depends directly on the ratio of SE to TG in the cell. Rogers et al. (4) found that supplementation with oleic acid (a fatty acid that stimulates TG synthesis) together with AGR decreased the SE/TG ratio, which in turn prevented LCL-LD formation. Similarly, complete genetic ablation of TG breakdown via lipolysis prevented LCL-LD formation. Thus, the authors demonstrated that the formation of LCL-LDs is dependent upon the ratio of SE to TG in the droplet, which increases when TGs are catabolized during glucose restriction.

How do the distinct biophysical properties of LCL-LDs impact their function? The authors found that the canonical LD protein Erg6 rapidly relocalizes to the ER from LDs in response to AGR, coinciding with an increase in the SE/TG ratio. Decreasing the SE/TG ratio with oleic acid blocked Erg6 ER localization. They hypothesized that Erg6 localization is dependent on the phase state of the neutral lipid core. In a clever experiment to directly test the dependence of Erg6 localization on the phase state of LDs without changing their lipid composition, they heated yeast cells to 40°C. They inferred this would revert SE-rich LDs from a liquid crystal to an amorphous liquid phase. Remarkably, Erg6 relocalized from the ER to LDs after only 8 min of heating. These observations were paired with studies in HeLa cells loaded with either oleic acid or cholesterol to form TG- or SE-rich LDs, respectively. LDs in cholesterol-loaded HeLa cells exhibited birefringence, an established property of SEs in a liquid crystal phase. The authors demonstrated that both Erg6 and Pln1 target more efficiently to TG-rich LDs than to SE-rich LDs.

The selective targeting of Erg6 and Pln1 to TG-rich LDs suggested that the physical properties of LCL-LDs may impact which proteins coat their surface. These data were consistent with previous reports of proteins that preferentially target either TG-rich or SE-rich droplets (10). Therefore, heterogeneity in the neutral lipids making up the core of the droplet may also affect the LD proteome. To characterize how glucose restriction changes LD surface proteins, the

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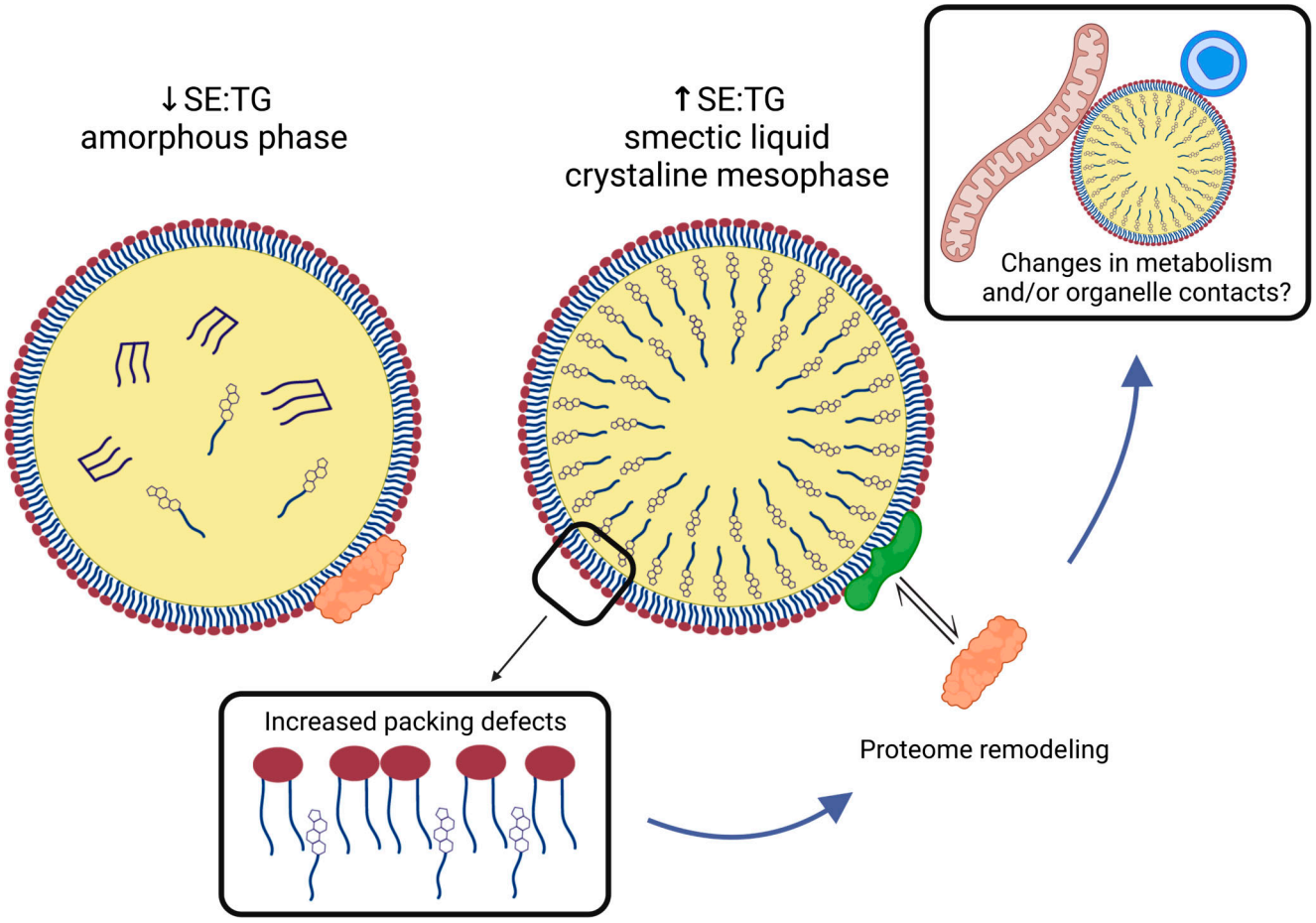

Figure 1. **LD cores with a high ratio of SE to TG form smectic LCLs.** Simulations of LCL-LDs suggest they have increased packing defects in the phospholipid monolayer, which could underlie observed remodeling of the LD proteome. These proteome changes are likely to cause changes in metabolism and/or membrane contacts with other organelles.

authors compared the proteomes of log-phase and AGR LD fractions and infranatants using mass spectrometry. They selected proteins that were specifically enriched in the LD fraction relative to the infranatant for further analysis. They imaged fluorescently tagged hits in yeast and measured their enrichment on LDs. In this screen, the authors identified several new proteins that, like Erg6, relocalize from LDs to the ER upon AGR. Other proteins, such as the TG lipases required for LCL-LD formation, retained their localization upon AGR. Moreover, Snx4, a vesicular trafficking protein not previously identified as an LD protein, was identified as a protein that binds to LDs upon glucose restriction. To probe how AGR might alter protein targeting, the authors conducted molecular dynamics simulations using lipid "trilayers" of neutral lipid and phospholipid to mimic LDs. Modeling a 3:1 ratio of SE:TG (the ratio present in AGR-treated yeast) causes the neutral lipid core to partition, with SEs enriched at the monolayer surface relative to TG. Cholesterol moieties of SEs and surface phospholipids preferentially interact, causing them to interdigitate at the monolayer surface and drive partitioning. Phospholipid/SE interdigitation causes monolayer packing defects, which may in turn alter protein affinity to the monolayer surface (Fig. 1).

This work raises many new avenues of investigation into the interplay between LD composition and function. First, apart from glucose restriction, what other conditions might trigger the formation of LCL-LDs? Based on this study, we predict that any condition that increases the SE to TG ratio within LDs could have this effect. It will also be interesting to learn whether LCL-LDs are more common in certain cell types or disease states. Second, how are the structural changes within LCL-LDs sensed by proteins? Are there specific motifs shared by classes of proteins that drive their recruitment to or relocalization from LCL-LDs? Finally, what are the physiological consequences of the proteome remodeling that occurs on LCL-LDs? Rogers and colleagues' (4) proteomics data suggest changes in LD-organelle membrane contacts and lipid metabolism (Fig. 1). It will be fascinating to explore the downstream effects of this proteome remodeling in different cell types, or in response to different developmental, environmental, and disease conditions that alter the SE/TG balance of LDs.

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References

1. Olzmann, J.A., and P. Carvalho. 2019. *Nat. Rev. Mol. Cell Biol.* https://doi.org/10.1038/s41580-018-0085-z
2. Welte, M.A., and A.P. Gould. 2017. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids*. https://doi.org/10.1016/j.bbalip.2017.07.006
3. Mahamid, J.D., et al. 2019. *Proc. Natl. Acad. Sci. U.S.A.* https://doi.org/10.1073/pnas.1903642116
4. Rogers, S., et al. 2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202205053
5. Engelman, D.M., and G.M. Hillman. 1976. *J. Clin. Investig.* https://doi.org/10.1172/JCI108554
6. Ioannou, G.N., et al. 2019. *Hepatol. Commun.* https://doi.org/10.1002/hep4.1348
7. Ginsburg, G.S., et al. 1984. *Prog. Lipid Res.* https://doi.org/10.1016/0163-7827(84)90002-X
8. Shimobayashi, S.F., and Y. Ohsaki. 2019. *Proc. Natl. Acad. Sci. U.S.A.* https://doi.org/10.1073/pnas.1916248116
9. Rogers, S., et al. 2021. *eLife*. https://doi.org/10.7554/eLife.62591
10. Khor, V.K., et al. 2014. *PLoS One*. https://doi.org/10.1371/journal.pone.0105047