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SPOTLIGHT

Seipin regulates the formation of nuclear lipid droplets from a distance

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Nuclear lipid droplets (nLDs) are poorly characterized outside of the liver. In this issue, Sołtysik et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202005026) show that seipin is absent from the nucleus but seipin deficiency promotes nLD formation by increasing nuclear phosphatidic acid.

Lipid droplets (LDs) are cellular organelles that regulate lipid homeostasis and many other cellular functions. To date, most studies have focused on cytoplasmic LDs (cLDs), which are derived from the ER. Although the molecular underpinnings of cLD biogenesis are not fully clear, a few proteins and lipids have been implicated (1). Seipin, an evolutionarily conserved, integral membrane protein of the ER, and phosphatidic acid (PA), a negatively charged conical lipid, regulate the formation of cLDs (2, 3, 4). Recent evidence indicates that seipin and its partner LDAF1 play a structural role in facilitating LD initiation from the ER (3), although other studies suggest that seipin may act on LD dynamics by directly regulating the synthesis and distribution of phospholipids, including PA (4, 5, 6).

LDs exist in the nucleoplasm, but few studies have investigated the biology of nuclear LDs (nLDs). Yeast cells appear to generate nLDs de novo from the inner nuclear membrane (INM), which has a distinct lipid composition and harbors enzymes for triacylglycerol (TAG) synthesis (7). Notably, there are dramatically increased nLDs in seipin-deficient yeast cells (7). In hepatocytes where there are abundant nLDs, Fujimoto and colleagues demonstrated that these nLDs are derived from ApoB-free luminal LDs (LLDs) and their formation depends on the ER luminal lipid transfer protein, microsomal triglyceride transfer protein (MTP; 8). The LLDs in the ER lumen of hepatocytes can intrude into the nucleoplasm through breaches of the INM, giving rise to nLDs. Whether and how other mammalian cells generate nLDs remain largely unexplored.

In this issue, Sołtysik et al. found that nLDs were frequently observed in non-hepatocytes, such as U2OS cells, a cell line of osteosarcoma origin (9). Importantly, U2OS nLDs are different from those found in hepatocytes, as their formation is not regulated by MTP. Using a probe (NLSx3-HPos) targeting initial LDs in the nucleus, the authors demonstrated that nLDs in U2OS cells originated from the INM, but not from ER luminal LDs. The number of nLDs increased when U2OS cells were cultured with oleate, a common form of fatty acid. Treating cells with triacsin C, a long-chain-fatty-acid–CoA ligase (ACSL) inhibitor, suppressed the oleate-stimulated formation of nLDs. These results indicate that the biogenesis of nLDs in U2OS cells depends on TAG synthesis. Consistently, the researchers observed the presence of key enzymes in TAG synthesis, including ACSL3, GPAT3/4, AGPAT2, lipin-1β, and DGAT1/2, in the nucleus; knockdown studies showed that ACSL3 and GPAT3/4 are essential for nLD formation. Wild-type lipin-1β, but not catalytically dead mutants, increased nLD counts. This is interesting because lipin-1β is known to translocate to the nucleus upon mTORC1 inhibition by Torin, a condition now shown to also increase nLDs (9). The authors further investigated if the formation of nLDs is regulated by seipin, a key protein for the biogenesis of cLDs (2). Seipin-deficient U2OS cells showed dramatically increased nLDs as well as more connections between nuclear LDs and the INM. Surprisingly, however, no seipin was detectable on the INM by bimolecular fluorescence complementation or by immunoelectron microscopy. Thus, seipin regulates nLD formation indirectly by functioning in another subcellular compartment. While the exact molecular function of seipin remains controversial, multiple lines of evidence suggest that seipin functions to regulate the level and distribution of PA, either by direct binding of PA and/or by inhibiting the activity of GPAT3/4 (Fig. 1; 4, 5, 6). Indeed, Sołtysik et al. found that the level of PA on the INM and nLDs increased significantly in seipin-deficient cells. Interestingly, the levels of lipin-1β mRNA and protein both increased by approximately fivefold in seipin-deficient cells, and knocking down lipin-1 reduced nLD formation in seipin-deficient cells. Thus, it appears that seipin deficiency increases nuclear PA, which subsequently recruits lipin-1β to promote TAG synthesis and the de novo formation of nLDs (Fig. 1).

Overall, these results convincingly demonstrate that increased TAG synthesis can

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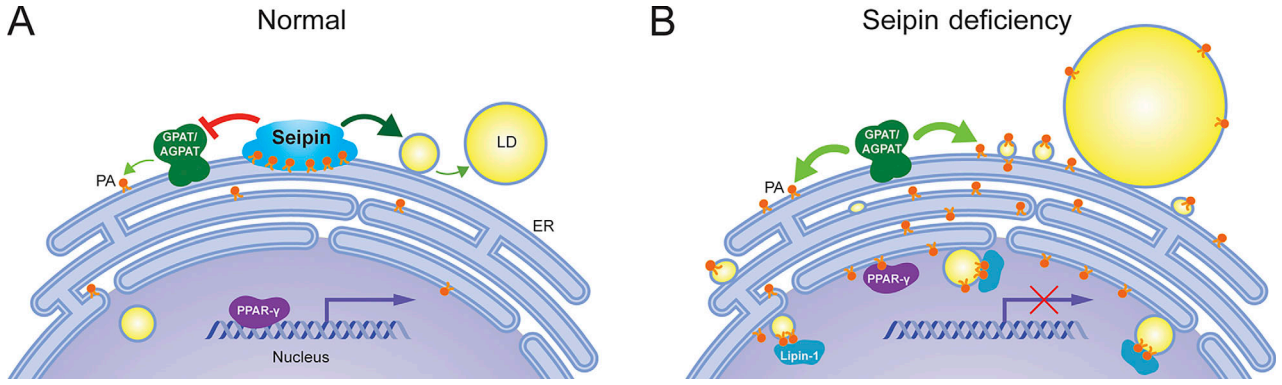

Figure 1. **Seipin regulates the formation of nLDs and adipogenesis through control of the level of PA in the nucleus. (A)** In normal cells, seipin localizes to the ER and restricts the level and distribution of PA by direct binding of PA and/or by inhibiting the activity of GPAT3/4. There is a limited amount of PA on the INM, and PPARγ drives the transcription of adipogenic genes during adipocyte differentiation. **(B)** In seipin-deficient cells, more PA is available in the ER due to increased GPAT activity and/or a lack of sequestration by seipin. The increased PA, a conical lipid that can impact surface/line tension of the ER, may hinder LD growth, leading to the formation of many small LDs. The increased PA may also keep LDs attached to the ER for a longer period of time, forming giant LDs. Upon seipin deficiency, more PA relocates to the INM, which would increase the number of nLDs, keep nLDs close to the INM, and trap PPARγ at the INM and nLDs, thereby blocking adipogenesis. Lipin-1β is recruited to the INM and nLDs by PA to promote TAG synthesis.

trigger the biogenesis of nLDs from the INM in nonhepatocytes, although nLDs are formed at a much slower rate than cLDs (9). The most interesting finding is that seipin can regulate the level of nuclear PA and the formation of nLDs despite its absence from the INM. This observation clearly demonstrates that seipin can impact nLD formation indirectly through PA and supports the notion that seipin's role in cLD formation may also be indirect (1, 4, 5). Nevertheless, more evidence is needed to firmly establish that nuclear PA is essential for increased nLD formation under seipin deficiency. For instance, methods that may reduce nuclear PA, such as overexpressing cytidine diphosphate DAG (CDP-DAG) synthase, can be employed to examine nLD formation in seipin-deficient cells. An intriguing observation is that the level of nuclear PA remains high in seipin-deficient cells, despite increased expression and nuclear localization of lipin-1β, a PA phosphatase. This suggests that the production and/or nuclear redistribution of PA triggered by seipin deficiency outweighs PA degradation by lipin-1β. The source of the increased PA requires additional work in the future. Increased GPAT activity and reduced PA sequestration by seipin may both contribute to elevated nuclear PA under seipin deficiency (Fig. 1).

These results also have profound implications for adipogenesis, a process centrally controlled by the transcription factor PPARγ (10). Seipin deficiency blocks adipogenesis and causes the most severe form of human congenital generalized lipodystrophy (CGL). It has long been puzzling how seipin, an integral membrane protein of the ER, can impact the nuclear activity of PPARγ. As PPARγ is regulated by lipid ligands, it was proposed that seipin deficiency may increase nuclear PA, which traps and antagonizes PPARγ (Fig. 1; 10). The results reported here lend strong support to this hypothesis. It is also interesting to note that Sołtysik et al. observed an increase in nuclear PA and nuclear translocation of lipin-1 upon mTORC1 inhibition by Torin. Inhibiting mTORC1 can block adipogenesis, and this effect may also arise from increased nuclear PA. Notably, lipin-1 deficiency and AGPAT2 deficiency also increase cellular PA, block adipogenesis, and cause severe CGL in mammals (10). Therefore, increased nuclear PA may represent a common cause of CGL.

Results from this study open important avenues for future research. Can increasing nuclear PA by other means drive nLD formation in mammalian cells? For instance, deficiency in CDP-DAG syntheses or AGPAT2 can increase cellular PA and block adipogenesis (4, 10). It would be worth examining nuclear PA and nLD formation when CDP-DAG synthase or AGPAT2 is compromised. DAG was implicated in nLD formation in yeast, and whether a similar role exists in mammalian cells requires further analyses (7). Importantly, recent insights into the biogenesis of nLDs in yeast and mammalian cells demand better characterization of how lipid species, including PA and DAG, may control the biogenesis of cytoplasmic LDs. Distinct from all other organelles, each LD is enclosed by a monolayer of lipids. Therefore, unlike COPII vesicles and autophagosomes, whose biogenesis from the ER requires dedicated protein machineries, the initiation and budding of cLDs/nLDs may be largely driven by local lipid composition of the ER/INM (1).

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