

COMMENTARY

Keeping FIT, storing fat: Lipid droplet biogenesis

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ABSTRACT

All eukaryotes store excess lipids in organelles known as lipid droplets (LDs), which play central roles in lipid metabolism. Understanding LD biogenesis and metabolism is critical for understanding the pathophysiology of lipid metabolic disorders like obesity and atherosclerosis. LDs are composed of a core of neutral lipids surrounded by a monolayer of phospholipids that often contains coat proteins. Nascent LDs bud from the endoplasmic reticulum (ER) but the mechanism is not known. In this commentary we discuss our recent finding that a conserved family of proteins called fat storage-inducing transmembrane (FIT) proteins is necessary for LDs budding from the ER. In cells lacking FIT proteins, LDs remain in the ER membrane. *C. elegans* has a single FIT protein (FITM-2), which we found is essential; almost all homozygous *fitm-2* animals die as larvae and those that survive to adulthood give rise to embryos that die as L1 and L2 larvae. Homozygous *fitm-2* animals have a number of abnormalities including a significant decrease in intestinal LDs and dramatic defects in muscle development. Understanding how FIT proteins mediate LD biogenesis and what roles they play in lipid metabolism and development are fascinating challenges for the future.

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Lipid droplets (LDs) are the major storage organelle for fat in most organisms.^{1,2} LDs play a key role in lipid homeostasis. However, recent findings suggest that LDs have many other functions; they play roles in protein degradation^{3,4} and the endoplasmic reticulum (ER) stress response,⁵ they act as sites for assembly of infectious virions,⁶ are involved in membrane trafficking and signal transduction, and act as a temporary storehouse of proteins.^{1,7,8} Understanding LD homeostasis, biogenesis and catabolism is critical for understanding the pathophysiology of lipid storage disorders like obesity, lipodystrophy (abnormal distribution of fat), type2-diabetes, insulin resistance, atherosclerosis and its associated diseases.^{9,10} These metabolic diseases have a wide range of crippling symptoms, the treatment options of which are few and not very effective.

We are still just beginning to understand the biogenesis of LDs. Before discussing what is known about this process, it is important to understand how LDs differ from other organelles. Most organelles are surrounded by a membrane bilayer that separates the

lumen of the organelle from the cytoplasm. In contrast LDs have a phospholipid monolayer that surrounds a core of neutral lipids.¹¹⁻¹⁴ LDs are surrounded by coat proteins; perilipins (PLIN) in mammals, and oleosins in plants, that regulate the access of lipid lipases to the neutral lipid core (for review see¹⁵⁻¹⁸). However, yeast and *C. elegans* lack LD coat proteins,^{19,20} suggesting that coat proteins are not necessary for LD formation or maintenance. Indeed, emulsions of neutral lipids and phospholipids without proteins form artificial LDs *in vitro*.

How do LDs form in cells? A number of models have been proposed but the simplest is one that has been termed the lens model. This model suggests that nascent LDs initially form blisters or lenses of neutral lipid that accumulate between the 2 leaflets of the bilayer membrane of the ER, where LD biogenesis occurs. As the lenses grow they eventually bud from the membrane and are then surrounded by a phospholipid monolayer¹¹⁻¹⁴ (Fig. 1). The elegant aspect of this model is that LD formation could be driven

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largely by the properties of neutral lipids in membranes and may not require any proteins other than the enzymes that synthesize neutral lipids. Neutral lipids are highly hydrophobic and therefore probably remain mostly in the hydrophobic core of the membrane after they are synthesized. It is thought that when the concentration of neutral lipids in a bilayer gets high enough, they spontaneously “oil out” and begin to form a lens between the 2 leaflets of the ER membrane. Obtaining evidence for this model has been difficult, the lenses are thought to be short lived and small, perhaps less than 100 nm.

To address this problem, we used a yeast strain in which nascent LD biogenesis can be controlled.²¹ This strain lacks 3 of the 4 neutral lipid-synthesising enzymes in yeast and has the fourth under a regulatable promoter. When the promoter is off, the cells are devoid of neutral lipids and LDs. However, when the promoter is activated, neutral lipid biosynthesis and LD biogenesis begins within minutes.²¹ We used this strain in conjunction with EM tomography to confirm that nascent LDs in fact form lenses in the ER membrane.²¹ Whether lens formation requires any proteins other than the enzymes that synthesize neutral lipids remains unknown.

The finding that nascent LDs form lenses in the ER membrane raises an interesting question: why do LDs bud into the cytoplasm and not into the lumen of the ER (at least in most cell types)? There are probably many factors that determine the directionality of LD

budding, including the shape of the ER membrane, the surface tension of the membrane, and the lipid composition of the ER. We have recently shown that a conserved family of proteins facilitates LD budding from the ER.²¹ These proteins, called fat storage-inducing transmembrane (FIT) proteins, are conserved and were first identified because, when over produced in mammalian cells, cause a dramatic increase in neutral lipid and LD production.²² Humans have 2 of these proteins, FIT1, which is expressed in muscle, and FIT2, found in all tissues.²² The yeast *S. cerevisiae* has 2 FIT2 homologs and lacks a homolog of FIT1. We found that in a yeast mutant lacking both FIT proteins, LDs fail to bud from the ER and often become wrapped by the ER membrane (Fig. 2). This function of FIT proteins is conserved since knock down of FIT2 in a human 3T3-L1 fibroblasts or the sole FIT protein in *C. elegans* (FITM-2) results in a similar cellular phenotype²¹ (Fig. 2).

How FIT proteins affect LD budding from the ER is not yet understood. It has been found that FITs bind triacylglycerides (TAGs) and diacylglycerol, a precursor of TAGs²³ and thus the FITs may play a role in lipid metabolism that somehow affects LD production. Indeed one of the 2 FIT proteins in yeast has been genetically implicated in lipid metabolism but its role is not known.²⁴

Yeast cells lacking both FIT proteins have only modest growth defects and have normal levels of neutral lipids and LDs. Interestingly, this is not the case in

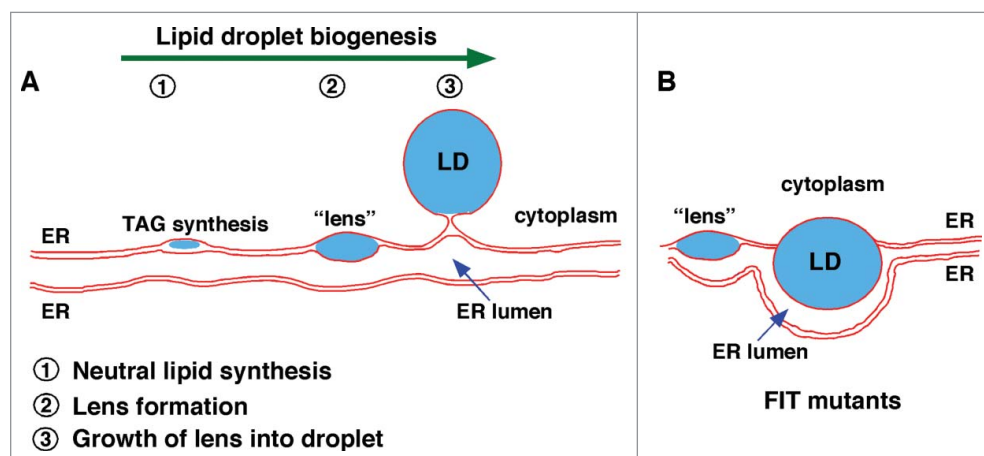


Figure 1. Model of LD biogenesis in wild-type cells and cells lacking FIT proteins. (A) Stages of LD biogenesis in wild-type cells. Neutral lipids are synthesized in the ER (1). When the amount of neutral lipid in the ER bilayer exceeds a threshold, lens-like structures form (2). Neutral lipid lenses grow and emerge out of the ER toward the cytoplasm, resulting in LDs consisting of a core of neutral lipids surrounded by a phospholipid monolayer. Mature LDs remain connected to the ER via membrane-bridge or separate from the ER. (B) In cells lacking FIT proteins LDs fail to bud from the ER and remain embedded in the ER membrane. They often also become wrapped by the ER membrane.

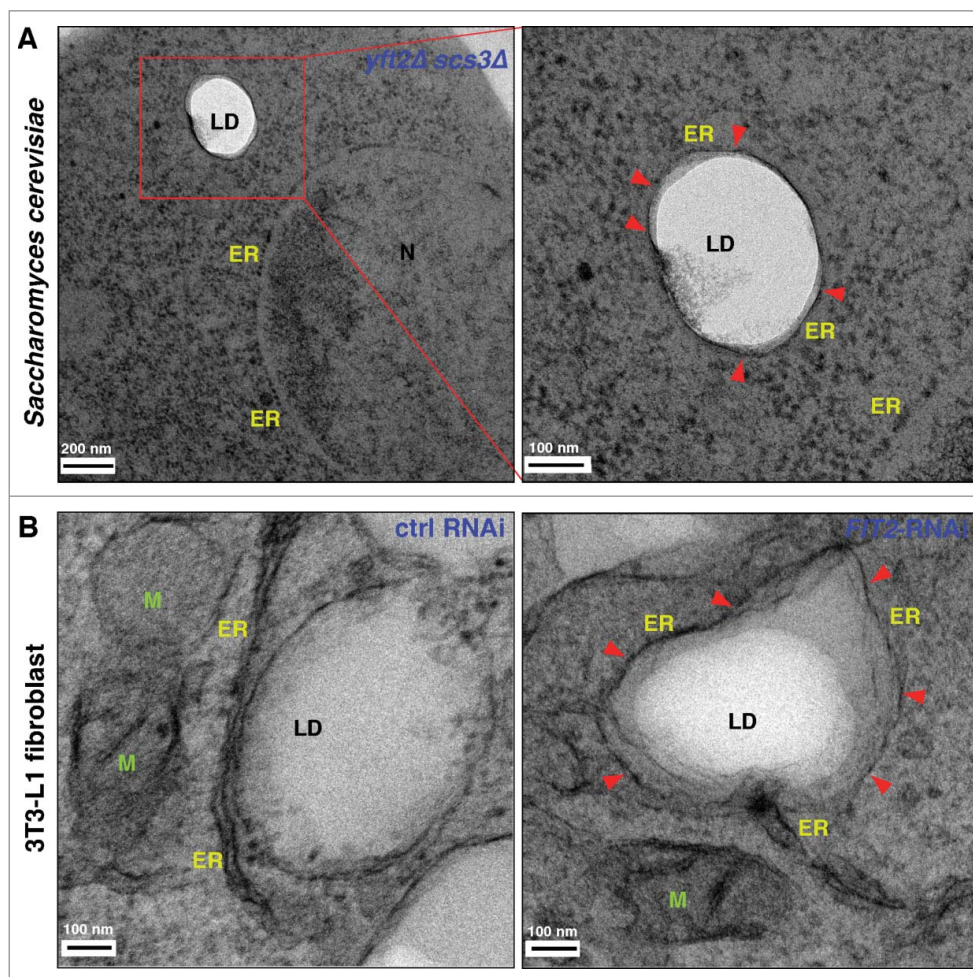


Figure 2. Lack of FIT proteins results in membrane wrapped LDs. (A) Yeast cells lacking FIT proteins (*yft2Δ scs3Δ*) were cryo-fixed using high pressure freezing and automatic freeze substitution and examined by electron microscopy. LDs are electron transparent. The ER membrane often wraps LDs in FIT mutants. Boxed region is shown in higher magnification. (B) 3T3-L1 fibroblast cells treated with control RNAi or FIT2-RNAi were subjected to chemical fixation and examined by electron microscopy. Arrowheads denote the ER membrane wrapping LDs. ER, endoplasmic reticulum; LD, lipid droplet; N, nucleus; M, mitochondria. Red arrowheads indicate ER membrane wrapping LDs.

metazoans, where elimination of FITs is lethal in mice and worms.^{21,25} Selective knockdown of FIT2 in adipose tissue renders mice lipodystrophic and insulin resistant.²⁶ Most homozygous *fitm-2* animals die as larvae; a minority of animals do survive to adulthood and give rise to viable embryos that die as L1 and L2 larvae.

Why FIT protein deletion is lethal in *C. elegans* is a fascinating mystery. It may be that a defect in LD budding from the ER disrupts ER function in *C. elegans* even though it does not in yeast. Lipoprotein production begins in the ER lumen in mammalian cells and probably in *C. elegans* as well. It could be that this essential process is disrupted by the presence of LDs in the ER membrane. We have found a significant reduction in the number and size of LDs in intestinal cells,²¹ suggesting that *fitm-2* animals have a profound

lipodystrophy. It may be that lipid uptake by intestinal cells is disrupted in these cells, but this remains to be determined. Alternatively, it may be that the FIT protein in *C. elegans* plays an essential role in lipid metabolism that is not shared by the yeast proteins. It should be noted that a mutant lacking one of the 2 yeast FIT proteins, called Scs3p, has been implicated in lipid metabolism but its role is not known.

It is also possible that *C. elegans* lacking *fitm-2* die because of a defect in muscle development. Remarkably, EM analysis of *fitm-2* animal revealed improper development of body wall muscle. Homozygous *fitm-2* worms showed very thin body wall muscle (average diameter 200–400 nm) compared to heterozygous *fitm-2/+* worms (1–1.2 μm) (unpublished observations). Consistent with this *fitm-2* worms showed

severe locomotion defects compared to heterozygotes, suggesting impairment in muscle development in these animals. Moreover *fitm-2* worms lacked LDs in the proximity of muscle fibers compared to *fitm-2/+* worms (unpublished observations).

FIT protein may play an important role in muscle development in mammals as well. Mammals have a muscle-specific FIT protein (FIT1) that seems to be absent in worms;²² it may be that the sole FIT protein in worms performs the functions of FIT1 and FIT2 in mammals. It has recently been found that the expression of the FIT1 gene is altered in patients with facio-scapulohumeral muscular dystrophy (FSHD, discussed in²⁷). FSHD is characterized by muscle weakness and wasting in the facial, shoulder and upper arm regions. The symptoms of FSHD appear in adolescence with a prevalence rate of 1 in 20,000 people. FIT1 shows regulated expression during development in the skeletal muscles of pigs with highest expression at 4 months of age and at day 2 of differentiation in C2C12 myoblasts.²⁷ MyoD1, a transcription factor that is crucial for myogenesis, transactivates the promoter of porcine FIT1 gene.²⁷ MyoD1 binding sites are conserved among pig, rat, mice and humans suggesting a similar mechanism of activation of the FIT1 gene.²⁷ The role of FIT proteins in muscle development and how lack of FIT proteins causes muscular dystrophy are important questions for future studies.

The observation that *fitm-2* mutants in *C. elegans* have defects in body wall muscle raises many questions about the primary defect in these animals. Is muscle development perturbed because of defects in lipid metabolism, lipid signaling, or LD formation? Future studies using genetic suppressor screens may help identify the other players in this process and may shed light on the molecular mechanism of specific muscular dystrophies. The larval lethality of these mutants suggests that other tissues are compromised as well. These phenotypes will be the focus of future investigations.

Disclosure of potential conflicts of interest

The authors declare no competing financial interests.

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