

Thematic Review Series: Lipid Droplet Synthesis and Metabolism: from Yeast to Man

Lipid droplets as fat storage organelles in *Caenorhabditis elegans*

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Abstract Lipid droplets are evolutionarily conserved organelles where cellular fat storage and mobilization are exquisitely regulated. Recent studies have defined lipid droplets in *C. elegans* and explored how they are regulated by genetic and dietary factors. *C. elegans* offers unique opportunities to visualize lipid droplets at single-cell resolution in live animals. The development of novel microscopy techniques and protein markers for lipid droplets will accelerate studies on how nutritional states and subcellular organization are linked in vivo. Together with powerful tools for genetic and biochemical analysis of metabolic pathways, alteration in lipid droplet abundance, size, and distribution in *C. elegans* can be readily connected to whole-animal energy homeostasis, behavior, and life span. Therefore, further studies on lipid droplets in *C. elegans* promise to yield valuable insights that complement our knowledge gained from yeast, *Drosophila*, and mammalian systems on cellular and organismal fat storage.—Mak, H. Y. Lipid droplets as fat storage organelles in *Caenorhabditis elegans*. *J. Lipid Res.* 2012. 53: 28–33.

Lipid droplets are ubiquitous fat storage organelles that are conserved in yeast, *C. elegans*, *Drosophila*, and mammals (1–3). They are also sites of regulated release of stored fat by lipases during cell growth and fasting (4). Therefore, lipid droplets are central to energy balance at cellular and organismal levels. Key structural and biochemical features of lipid droplets have been defined, and it is generally accepted that neutral lipids such as triglycerides (TAG) and cholesterol esters (CE) are stored in the interior of the organelle that is delimited by a phospholipid monolayer (5–7). Current models suggest that fatty acid influx can be accommodated by neutral lipid synthesis and a concomitant change in lipid droplet size or number in a tissue-specific manner (1, 8). For example, differentiation of mammalian white adipose cells is characterized by a decrease in lipid droplet number and an increase in lipid

droplet size until a dominant unilocular lipid droplet remains (9, 10). The mechanisms that govern lipid droplet size and number in white adipose cells and other cell types are not fully understood.

To study regulators of fat storage and lipid droplet size in metazoans, most studies have focused on the use of tissue culture cells and overexpressed lipid droplet-associated proteins or vital dyes as markers. These studies yielded important insights into how conserved proteins and signaling pathways are coupled to control cellular fat storage. Nevertheless, extension of observations made in tissue culture cells into whole-animal models is often time consuming and can lead to surprising results. In the last few years, *C. elegans* has emerged as an attractive model for studying fat storage in vivo (11, 12). Although *C. elegans* lacks dedicated adipose tissues, its intestine serves as the main site for fat storage. Yolk lipoproteins are synthesized in the intestine, exported into the body cavity (pseudocoelomic space), and taken up by developing oocytes (13–15). This is remarkably similar to yolk deposition in chicken, where yolk is synthesized in the liver and transported to the ovum via the bloodstream (16). Therefore, the *C. elegans* intestine may be viewed as a multifunctional organ that fulfills the roles of the liver and adipose tissues.

Inspection of the sequenced genome of *C. elegans* and functional studies through analysis of genetic mutants revealed extensive conservation of genes involved in fatty acid synthesis, elongation, desaturation, and degradation (17–23). Furthermore, fat storage in *C. elegans* appears to be controlled by conserved insulin, TGF- β , serotonin, and mammalian target of rapamycin (mTOR) signaling pathways (24–29). A number of reverse genetic techniques will accelerate functional dissection of signaling and protein interaction networks (30–32). The development of novel

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Abbreviations: ATGL, adipose triglyceride lipase; GFP, green fluorescent protein; LRO, lysosome-related organelle.

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transgenic technologies has also enabled expression of native or heterologous proteins to near-physiological levels (33). This is critical for structure-function analysis of specific proteins in vivo and the accurate targeting of fluorescent protein markers to lipid droplets and other subcellular organelles. The use of *C. elegans* offers a unique advantage of imaging lipid droplets in live animals at single-cell resolution. Their intestinal cells support lipid droplet expansion to >10 μm in diameter, similar to lipid droplets found in mammalian adipocytes (34). In contrast, adherent tissue culture cells have limited depth, which restricts spherical expansion of lipid droplets. Finally, *C. elegans* offers ample opportunities to connect alteration in cellular fat storage to whole-animal physiology, behavior, and life span (18, 35, 36). This review will focus on the discovery of lipid droplets in *C. elegans*, the methods to detect lipid droplets, and the proteins that associate with lipid droplets in this model organism. Thorough understanding of these areas will no doubt provide answers to fundamental questions regarding how lipid droplets accommodate changes in the cellular demand and supply of fat in an evolutionarily conserved manner.

IDENTIFICATION OF LIPID DROPLETS IN *C. ELEGANS*

In the absence of a specialized fat storage tissue, *C. elegans* store their fat primarily in the intestine. It is remarkable that the entire intestine is composed of only 20 cells, arranged in rings of two or four cells that enclose the lumen, which spans almost the entire length of the animal (~ 1 mm) (37, 38). Each intestinal cell is derived from a single embryonic blastomere; cell division ceases and organogenesis is complete before the hatching of a free-living animal (37, 38). Nevertheless, the intestinal cells increase in size that matches the growth of the animal as it progresses through four larval stages before maturing into a reproductive adult.

One of the defining features of lipid droplets is the phospholipid monolayer that encloses their neutral lipid core, as demonstrated by transmission and freeze-fracture electron microscopy (5–7). This sets lipid droplets apart from all other intracellular organelles that are bound by a phospholipid bilayer. Extensive ultrastructural studies have been performed in *C. elegans* that revealed numerous vesicular compartments of similar size in the intestinal cells (37–39). These compartments may be differentiated by their electron densities. Two putative fat and lipoprotein storage compartments have been proposed: electron-lucent lipid droplets and electron-opaque gut granules (37–40). Gut granules have also been detected using light microscopy and were subsequently defined as lysosome-related organelles (LRO) (41, 42). The appearance and relative abundance of these compartments are dependent on the developmental stages of the animal. However, membrane structures that surrounded these compartments were not studied in detail until recently.

To positively identify lipid droplets using electron microscopy in *C. elegans*, Zhang et al. took advantage of a mutant that appeared to undergo selective expansion of fat storage compartments (34) (Fig. 1). The *daf-22*/thiolase gene encodes the terminal enzyme in a peroxisomal β -oxidation pathway. DAF-22/thiolase was originally identified as a key enzyme for processing the fatty acid moiety of the *C. elegans* dauer pheromone (43). Loss of DAF-22/thiolase function in *C. elegans* specifically increases the level of triglycerides that are accommodated by expanded fat storage compartments in the intestinal cells (34). As most other vesicular structures are <2 μm in diameter, electron-lucent, grossly expanded compartments (>10 μm in diameter) could be readily identified by size. Indeed, it was found that such compartments were delimited by a phospholipid monolayer, thus confirming the existence of lipid droplets in *C. elegans* (34). Additional support came from the targeting of the adipose triglyceride lipase (ATGL) ortholog ATGL-1 to the surface of expanded lipid droplets in *daf-22* mutant animals (34). Since the initial report,

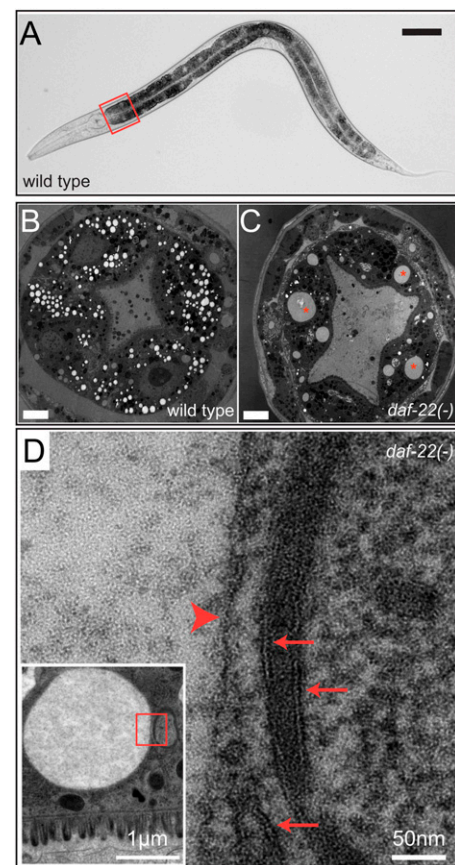


Fig. 1. Lipid droplets in *C. elegans* intestinal cells. A: A wild-type larval stage L4 animal. The first intestinal segment is boxed in red. Scale bar, 100 μm . B, C: Electron micrographs showing cross-sections of the first intestinal segment in wild-type (B) and *daf-22*(-) (C) mutant animals. Red asterisks indicate enlarged lipid droplets. Scale bar, 5 μm . (D) Electron micrograph showing a phospholipid monolayer (red arrowhead) that surrounds an expanded lipid droplet (boxed area in inset) in contrast to phospholipid bilayers (red arrows) of nearby organelles. The lumen of the intestine is at the bottom of the inset. B–D: Reprint from Ref. 34.

lipid droplets have also been identified in wild-type animals by transmission electron microscopy (40). It was also determined that lipid droplets are distinct from LROs (40), a compartment that was previously proposed to store fat in *C. elegans* (44). Furthermore, expanded lipid droplets have been reported in mutant animals that lack ACS-3/acyl-CoA synthetase (45).

VISUALIZATION OF FAT STORAGE IN *C. ELEGANS*

Attempts to visualize and quantitate fat storage long preceded the definitive identification of lipid droplets in *C. elegans*. Sudan Black has been widely adopted as a stain for intracellular fat since its first usage on bacteria (46). In *C. elegans*, animals that expressed a defective insulin/IGF receptor ortholog DAF-2 showed more intense Sudan Black staining than wild-type animals (29). Subsequent biochemical measurement confirmed that loss of insulin signaling elevated triglyceride levels in *C. elegans*, validating Sudan Black staining as a method for visualization of fat storage (47). Sudan Black staining was most prominent in intestinal cells, thus giving rise to the notion that the intestine is the major fat storage organ in *C. elegans*. In addition, Sudan Black staining was observed in the hypodermis and gonad, suggesting that fat is exported from the intestine to other tissues. Although Sudan Black staining was widely employed in other studies (27, 48–50), the fixation protocol was not readily adaptable for large-scale genetic and functional genomic screens. To overcome this, Nile Red and BODIPY-conjugated fatty acid were used as alternatives for fat staining in live animals (47). Nile Red was first introduced as a fluorescent vital stain for intracellular lipid droplets in mammalian cells (51), whereas BODIPY-conjugated fatty acid had been used to monitor fatty acid uptake in mammalian cells (52). It was assumed that worms would ingest Nile Red or BODIPY-conjugated fatty acid from *E. coli* that had been grown in the presence of these dyes on agar plates. This was followed by absorption of the dyes through the intestinal epithelium and their accumulation in fat storage compartments in intestinal cells. The precise mechanisms of absorption and turnover of these dyes are currently unknown.

Nile Red staining was initially used to conduct a whole-genome RNAi screen, which resulted in the discovery of hundreds of gene inactivations that altered the intensity and/or pattern of staining within intestinal cells (47). Many genes with conserved function in fat metabolism were uncovered, suggesting that Nile Red staining might serve as an indicator of fat storage in *C. elegans*. However, recent evidence suggests that vital staining with Nile Red and BODIPY-conjugated fatty acid also illuminates LROs, and that their accumulation in lipid droplets is highly dependent on genetic backgrounds (40, 44, 53–56). Therefore, future use of Nile Red as a vital dye for monitoring fat storage in *C. elegans* should always be validated with biochemical measurement of fat content. Further refinement may include analysis of the emission spectrum of Nile Red, which differs in lipid droplets versus lysosome related organelles (40, 45). This is because Nile Red in a neutral

lipid environment (i.e., lipid droplets) will emit yellow-gold fluorescence instead of red fluorescence (51). Note that Nile Red, BODIPY, or Oil-Red-O staining on fixed *C. elegans* samples appears to faithfully highlight lipid droplets and reflect biochemical measurements of fat content (40, 54–57).

The continual demand for identification of fat storage compartments in live samples has led to the development and implementation of multiple label-free lipid imaging techniques (58–60). The vibrations of C-H bond in lipids allow sensitive, label-free detection of lipid droplets using coherent anti-Stokes Raman scattering (CARS) microscopy in mammalian cells (59). This method was subsequently used to visualize fat storage compartments in live *C. elegans* (54, 61, 62). By exploiting vibrational characteristics of C = C double bonds, CARS microscopy also allowed the detection of changes in relative abundance of unsaturated fatty acids in mutant *C. elegans* that lacked specific fatty acid desaturases (62, 63). More recently, stimulated Raman scattering (SRS) microscopy has been introduced (58). Compared with CARS microscopy, SRS microscopy was reported to be more sensitive and less prone to background noise from structures unrelated to lipids. It was demonstrated in *C. elegans* that SRS microscopy could be used to visualize intracellular fat storage compartments and measure organismal fat content quantitatively (64). Using SRS microscopy, 272 gene inactivations by RNAi have been surveyed, which yielded 9 potentially new regulators of fat content in *C. elegans* (64). Although vesicular structures detected by CARS or SRS microscopy in *C. elegans* are likely to be lipid droplets as in mammalian cells, simultaneous imaging of fluorescent protein markers would be a logical step to determine if these label-free imaging techniques detect all lipid droplets.

LIPID DROPLET-ASSOCIATED PROTEINS IN *C. ELEGANS*

A large number of lipid droplet-associated proteins have been identified from proteomics studies using mammalian and *Drosophila* cell and tissue extracts (65–68). In contrast, similar proteomics studies have not been carried out in *C. elegans*. This is in part because existing protocols for large-scale protein extraction and purification using frozen worm samples is incompatible with lipid droplet preservation (Dawn Brasaemle; personal communication). Intriguingly, the Perilipin and CIDE families of proteins, which have been widely used as reference markers for lipid droplets in other organisms, are absent in *C. elegans*. Therefore, few *C. elegans* proteins have been reported to be genuinely associated with lipid droplets. A strategy forward will involve selecting conserved proteins from mammalian or *Drosophila* lipid droplet proteomes and interrogate their localization in *C. elegans*. It may be prudent to concentrate on proteins that have a clear role in fat metabolism or regulation of lipid droplet size or number. One such candidate is ATGL-1. The *C. elegans* ATGL-1

lipase is crucial for the balance of fat utilization and preservation in the developmentally arrested dauer larval stage (69). Long term (>1 month) survival of dauers is dependent on tight regulation of ATGL-1 activity by the AMP kinase (69). In *Drosophila*, the ATGL ortholog Brummer, when fused to green fluorescent protein (GFP), has been shown to localize to the lipid droplet surface (70). On the basis of these results, it was satisfying to observe that the *C. elegans* ATGL-1::GFP fusion protein was also localized to the lipid droplet surface, both in wild-type and mutant animals with enlarged lipid droplets (40, 45). By coupling confocal microscopy with spectral analysis and linear unmixing procedures, it was further demonstrated that ATGL-1::GFP-marked lipid droplets are distinct from the autofluorescent lysosome related organelles (Fig. 2) (34, 40). Fat mobilization from lipid droplets is a highly regulated process that involves not only adipose triglyceride lipase but also its activator CGI58 and hormone-sensitive lipase (4). Therefore, the *C. elegans* orthologs of CGI58 and hormone-sensitive lipase are prime candidate markers for lipid droplets in future studies.

The absence of Perilipin and CIDE families of proteins warrants further discussion. The Perilipin family members are found in mammals and invertebrates, including *Drosophila* and *Dictyostelium* (71). However, no Perilipin orthologs have been identified through sequence homology in *C. elegans* or other nematode species. As *Dictyostelium* is more primitive than nematodes, it is plausible that the Perilipin family was lost during evolution. Perilipin is known to play an important role in basal and hormone-stimulated lipolysis through its association with the lipid droplet surface and its stimulatory activity toward ATGL and hormone-sensitive lipase (HSL) (72–77). The absence of Perilipin in *C. elegans* gives rise to an intriguing possibility that additional Perilipin-independent regulatory mechanisms may exist for ATGL- and HSL-mediated lipolysis. Alternatively, proteins that share structural and functional similarity with Perilipin may have greatly diverged in the primary sequence that precludes their discovery through homology searches.

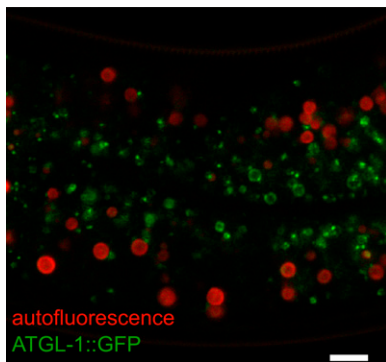



Fig. 2. ATGL-1::GFP as a marker for lipid droplets in *C. elegans* intestinal cells. Confocal microscopy and linear unmixing distinguishes lipid droplets that are marked by ATGL-1::GFP fusion protein (in green) and autofluorescent LROs (in red). Scale bar, 10 μ m.

In contrast to the Perilipins, the CIDE family proteins are found only in vertebrates (78). All three family members, CIDEA, CIDEB, and CIDEc, have been shown to associate with lipid droplets, and they play important roles in regulating whole-body fat storage and energy balance in mice and human (79–86). Ectopic overexpression of CIDE proteins modulates lipid droplet size (82, 83). One may speculate that the CIDE proteins have evolved in vertebrates to add an additional level of regulation for fat storage in lipid droplets. It is also plausible that divergent proteins substitute for CIDE protein functions in *C. elegans* and other invertebrates.

PERSPECTIVES

With the definitive identification of lipid droplets as fat storage organelles in *C. elegans*, now is the time to harness the forward and reverse genetic, biochemical, and imaging tools available to study the cell biology of fat storage and mobilization in this well-established model organism. Because the diet of *C. elegans* can be easily manipulated by feeding with different *E. coli* strains, analysis of lipid species and other metabolites will shed light on how dietary factors affect fat storage in lipid droplets (34, 56, 87, 88). Cell biological studies can also be coupled with powerful analytical methods that allow simultaneous monitoring of fatty acid absorption, elongation, and de novo synthesis in *C. elegans*, which have yet to be employed in other metazoans (89). Although the intestinal cells are the major site of fat storage in *C. elegans*, with the advent of label-free imaging techniques and fluorescent protein markers for lipid droplets, it will be of great interest to determine whether the regulators of fat storage in intestinal cells function in a similar fashion in tissues such as the hypodermis and gonad. It is plausible that additional mechanisms may operate in these tissues to support rapid turnover of fat for energy and reproduction. For example, small lipid droplets provide a larger surface area-to-volume ratio that favors lipolysis. The size of lipid droplets in the hypodermis and gonad of *C. elegans* may, therefore, be tightly regulated.

The unique advantage of live animal imaging at single-cell resolution throughout the life cycle should see the use of *C. elegans* in addressing additional fundamental questions on lipid droplets. How is the interaction between lipid droplets and other subcellular organelles, such as the endoplasmic reticulum and mitochondria, maintained? What is the functional consequence of such interactions? Are the distribution and movement of lipid droplets regulated in different developmental stages and nutrient levels? Careful implementation of genetic, biochemical, and microscopy techniques should yield insights that can be readily extended from *C. elegans* to mammals. 

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