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C. elegans Fat Storage and Metabolic Regulation

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Abstract

C. elegans has long been used as an experimentally tractable organism for discovery of fundamental mechanisms that underlie metazoan cellular function, development, neurobiology, and behavior. *C. elegans* has more recently been exploited to study the interplay of environment and genetics on lipid storage pathways. As an experimental platform, *C. elegans* is amenable to an extensive array of forward and reverse genetic, a variety of “omics” and anatomical approaches that together allow dissection of complex physiological pathways. This is particularly relevant to the study of fat biology, as energy balance is ultimately an organismal process that involves behavior, nutrient digestion, uptake and transport, as well as a variety of cellular activities that determine the balance between lipid storage and utilization. *C. elegans* offers the opportunity to dissect these pathways and various cellular and organismal homeostatic mechanisms in the context of a genetically tractable, intact organism.

Introduction

Many core metabolic pathways found in mammals are well conserved in *C. elegans*. These include pathways for fatty acid synthesis, elongation and desaturation, mitochondrial and peroxisomal β -oxidation of fatty acids, glycolysis, gluconeogenesis and amino acid metabolism [1–6]. Additionally, counterparts of a number of pathways central to regulation of mammalian metabolism appear to have similar function in *C. elegans* metabolism. Examples include fat and sugar transporters, nuclear hormone receptor and SREBP (sterol response element binding protein) transcriptional regulators, energy-sensing kinases such as AMP-activated kinase (AMPK) and TOR kinase, as well as neuroendocrine regulators such as insulin and serotonin ([6] and references therein).

C. elegans fat regulation differs from mammals in a number of aspects. There are no mesoderm-derived adipose cells dedicated specifically for fat storage in *C. elegans*. These animals store fat primarily in their intestinal and skin-like epidermal cells (see below). *C. elegans* also lack certain key mammalian fat regulatory mechanisms. For example, leptin, which is a core adiposity signal arising from adipose cells in mammals, has no sequence identifiable homologue in *C. elegans*. Experimental evidence, however, suggests the existence of signaling mechanisms that communicate between peripheral sites of fat storage/utilization and neural sites in homeostatic regulation of energy balance [7]. Other key differences include the fact

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that *C. elegans* are cholesterol auxotrophs, and must obtain cholesterol from their diet. Since very small dietary quantities of cholesterol are sufficient for *C. elegans* viability, it is thought cholesterol is not a required component of *C. elegans* cell membranes, but rather is necessary only for sterol-based signaling [8]. Moreover, unlike mammals, *C. elegans* are not dependent on dietary supplies of essential fatty acids (C18:2n6 and C18:3n3) as they have the set of desaturases and elongases necessary for synthesis of these lipids [9]. Despite the noted differences, the deep evolutionary conservation of key metabolic pathways and their regulators suggest that analyses of lipid storage pathways in *C. elegans* should be broadly informative. In support of this assertion, several newly identified *C. elegans* fat regulatory pathways have already been shown to also regulate mammalian lipid storage [5,10,11].

The field of *C. elegans* fat biology is very much in its infancy. While *C. elegans* convert excess energy into triglycerides that are stored in distinct droplet-like structures, their membrane and biophysical characteristics, associated proteins, and the extent of heterogeneity in the biochemical composition of their stored contents are largely undefined. Therefore, the aim of this review is to provide a concise overview of *C. elegans* fat storage and molecular mechanisms known to regulate the influx and efflux of lipids in these animals. Along the way, we highlight experimental approaches as well as similarities and differences in the regulation of lipid metabolism in *C. elegans* and mammals. Additional detailed discussion of pathways mentioned in this review can be found in references [6] [12] and [13].

Tissue and subcellular characterization of *C. elegans* lipid stores

C. elegans have a relatively simple body plan. Adult organs and tissues are comprised of an epithelial system that includes skin-like epidermal cells, a nervous system, excretory system, muscle, scavenger cells, reproductive system and sexual organs, alimentary tract consisting of the pharynx, the *C. elegans*' feeding organ, intestine and rectum. These organs are arranged within the cylindrical body shape of the animal with the alimentary tract and the gonad forming an inner tube encapsulated by an outer tube consisting of all the other tissues. These inner and outer tubes are separated from each other by a fluid-filled cavity through which secreted molecules such as insulins and lipid-laden lipoprotein-like molecules reach various tissues.

C. elegans feed on bacteria by the pumping action of the pharynx, where bacteria are physically disrupted and propelled into the lumen of the intestine. Intestinal cells are columnar polarized epithelial cells whose apical membranes face the intestinal lumen. The apical surface of intestinal cells contains microvilli necessary for absorption of nutrients. Neighboring intestinal cells are tightly coupled by apical junctions that isolate the contents of the intestinal lumen, preventing spillover of its contents into the rest of the animal. As the only cell type with access to the intestinal lumen, these cells are also responsible for secretion of digestive lipases and peptidases [14,15]. For instance, the *C. elegans* genome encodes eight member of the α/β hydrolase lipase family. Of these eight, seven have predicted signal peptides, suggesting they may be secreted into the lumen and function as digestive enzymes. The mammalian gastric and pancreatic lipases are secreted member of the α/β hydrolase lipase family [16].

In addition to their role as an epithelial barrier, and in digestion and absorption, *C. elegans* intestinal cells carry out functions that are reminiscent of the drug detoxification activity of mammalian hepatocytes as well as the fat storage capacity of adipocytes. Additionally, the skin-like epidermal cells also function as a key fat storing tissue. A variety of lipid biosynthetic and degradation enzymes are expressed in intestinal and epidermal cells. For example, enzymes of the acyl-CoA synthetase, carnitine palmitoyl transferase, elongase, enoyl-CoA hydratase 3-hydroxy acyl-CoA dehydrogenase and acyl-CoA oxidase families have been shown to be expressed in the intestine, epidermis or both [4,6,7,14]. Consistent with these expression patterns, analyses of serial sections of *C. elegans* by electron microscopy (EM) reveal

substantial lipid-like depots in intestinal and skin-like epidermal cells [17] However, identification of these depots as actual lipid-storing subcellular compartments awaits experimental verification. Biochemical fractionation of total triglyceride pools extracted from *C. elegans* has revealed that, similar to mammals, these pools contain a range of saturated, monounsaturated, and polyunsaturated fatty acids [4,9,18–21]. However, whether intestinal and epidermal cells have a similar composition of stored fat is not known.

Lipid staining dyes Sudan Black and Nile Red and fluorescently (e.g. BODIPY) labeled fatty acids have been used to visualize stored lipids in either fixed or living animals. These dyes stain droplet-like structures primarily in intestinal and epidermal skin-like cells (Figure 1). Mutations or RNAi-mediated inactivation of *C. elegans* counterparts of genes that encode various lipid biosynthetic pathways or their transcriptional regulators cause substantially reduced levels of staining [5,11,19,20] while loss of function mutations in a variety of other genes such as *C. elegans* counterparts of some mammalian obesity genes [22,23] or a nuclear hormone receptor regulator of fat oxidation genes [4] cause increased staining.

Recently, coherent anti-Stokes Raman scattering (CARS) microscopy has been used to monitor *C. elegans* lipid stores [24]. This methodology allows visualization of fat stores without the need for invasive techniques such as fixation or dependence on fluorescent reporters, each of which have limitations [24]. In wild-type animals, intestinal fat stores visualized by Nile Red staining correspond well to the stores visualized by CARS microscopy. However, accumulation of epidermal fat, which can escape detection by Nile Red, is readily detectable by CARS microscopy [24] and staining with bodipy-conjugated fatty acids.

Although mutagenesis and genome-wide RNAi studies have identified hundreds of genes regulating intestinal and epidermal fat depots as gauged by Nile Red fluorescent intensity or Sudan Black staining, little is known about the membrane characteristics or molecular identity of proteins that localize to *C. elegans* fat depots. A number of the best-characterized mammalian lipid droplet associated proteins have no obvious *C. elegans* homologues. These include PAT family members perilipin, ADRP TIP-47 and S3–12. By contrast, *C. elegans* has homologues of seipin (R01B10.6), leipin (H37A05.1) caveolin (T13F2.8 and C56A3.7), CGI-58 (C25A12.1 and C37H5.3), and multiple rab proteins, suggesting that some aspects of mammalian lipid droplet metabolism are likely conserved in *C. elegans*.

Hermann and colleagues have proposed that lysosome-related, terminal endocytic compartments known as gut granules, are cellular sites of *C. elegans* intestinal fat storage [25]. These autofluorescent granules have a lipid bi-layer, are acidified and contain a vacuolar proton pump. Since these organelles do not contain LMP-1, the *C. elegans* LAMP homolog and a marker of lysosomes, they have been classified as lysosome related organelles [25,26]. The vital lipid dye Nile Red and BODIPY conjugated fatty acids both label these intestinal granules, and mutations that disrupt these organelles lead to a reduction or elimination of these stains [25]. However, these mutants have relatively normal triacylglyceride content and developmental rates, suggesting that loss of this compartment does not eliminate lipid storage [25]. Epidermal cells, which contain substantial fat depots, do not contain the autofluorescent gut granules. Thus, whether intestinal and epidermal cells rely on different subcellular mechanisms for fat storage is not known. One possibility is that the lysosome related organelles contain dietary fats taken up by intestinal cells. Similarly, whether intestinal fat is solely stored in these endocytic compartments, or whether vital dyes can only get access to these specific compartments is unresolved. Hermann and colleagues have suggested that *C. elegans* fat storage in intestinal cells may be analogous to lamellar bodies, which are fat containing lysosome-related organelles that function in storage and release of lung surfactants [27].

C. elegans lipid stores are dynamically regulated

C. elegans fat depots are dynamic. During normal development fat droplets increase both in size and number [24]. Interestingly, CARS microscopy volume measurements indicate that intestinal fat tends to accumulate in larger-sized droplets relative to small-sized droplets in epidermal cells. The number, size, and distribution of these droplets also change dramatically in response to environmental signals. For instance, in response to unfavorable environmental conditions, early larval stage animals enter an alternate developmental program known as the hibernating dauer state, which is characterized by accumulation of fat reserves and a subsequent shift in metabolism to favor utilization during extended periods starvation [1,3,28]. Accordingly, hibernating dauers display a greater number and density of fat droplets visualized by staining [29] and CARS microscopy methods [24]. Similarly, during the adult stage, changes in diet or food deprivation elicit changes in fat composition [20,30], expression patterns of metabolic genes [4,30,31], as well as changes in size and distribution of droplets (Ashrafi lab, unpublished observations). While a number of gene inactivations have been identified that alter *C. elegans* fat content, the molecular mechanisms that underlie biogenesis of droplet-like fat stores and regulation of their size, number, and distribution are largely unknown.

As in mammals, the balance between *C. elegans* fat storage and fat utilization pathways is under a variety of complex transcriptional, translational, and post-translational regulatory mechanism (reviewed in [6]). For instance, loss of function mutations as well as RNAi knockdown of *shp-1*, the *C. elegans* homolog of mammalian SREBP, cause dramatically reduced levels of fat storage as gauged by EM, biochemical and staining methods [5,11,19]. A number of mammalian SREBP transcriptional targets are also regulated by *shp-1*, including acetyl-CoA carboxylase, ATP-citrate lyase, fatty acid synthase, glycerol 3-phosphate acyltransferase and malic enzyme [5,11]. In another example, *nhr-49*, a *C. elegans* nuclear hormone receptor, regulates pathways that are analogous to those under regulation of mammalian peroxisome proliferator-activated receptors (PPARs) [4]. Inhibition of *nhr-49* in *C. elegans*, and PPAR family members in mice, leads to increased fat storage in these organisms, respectively [4,32,33]. PPARs and *nhr-49* regulate transcription of homologous targets, including genes involved in fatty acid β -oxidation, lipid binding and fatty acid desaturation [4,34].

Mobilization of stored fat is ultimately dependent on flux of lipids through β -oxidation pathways. The *C. elegans* genome encodes the full complement of peroxisomal and mitochondrial β -oxidation pathway components. For many of these components, multiple family members are encoded. Initial characterization of these genes suggests that different family members likely function under different conditions. For instance, as in mammals, increased neural serotonin signaling causes fat reduction in *C. elegans*. This is dependent on activity of a specific subset of mitochondrial and peroxisomal β -oxidation genes that are transcriptionally upregulated in response to neural serotonin signaling [7]. Other β -oxidation pathway components, which when inactivated cause fat accumulation, do not appear to play a role in serotonergic mobilization of fat stores [7]. Similarly, while both serotonin and *nhr-49* affect *C. elegans* fat, in part, through β -oxidation pathways, they do so via different β -oxidation family members [4,7]. It may be that the complexity and redundancy of fat oxidation pathways reflects the heterogeneity of various fat storage depots.

Flux of stored lipids through β -oxidation pathways is dependent on liberation of fatty acids from triacylglycerides through enzymatic activity of lipases. A number of well-studied mammalian lipases have close homologues in *C. elegans*, including hormone sensitive lipase (C46C11.1, for additional information see www.wormbase.org), and phospholipase A2 (C07E3.9 and C03H5.4). Desnutrin/adipose triglyceride lipase (ATGL) is a recently identified mammalian lipase. This enzyme contains an N-terminal patatin-like domain, and its expression

is highest in adipose tissue [35]. *C. elegans* has 3 homologous genes (C05D11.7, B0524.2 and D1054.1). Two recent studies have revealed a role for lipases in regulation of lipid storage in response to physiological perturbations. The *C. elegans* ATGL homologue C05D11.7 is regulated by AMP-activated kinase to modulate lipid mobilization during the alternative developmental dauer stage [36]. A second study found that the *C. elegans* lipase K04A8.5 acts downstream of signals from the germline to regulate fat storage [37].

Fatty acid uptake, transport and lipoproteins

Fatty acids can enter cells both through transporter independent diffusion across the cell membrane, as well as by active transport. Mammalian cells take up long chain fatty acids (LCFA) primarily via active transport [38]. Fatty acid transport proteins (FATPs) as well as the protein CD36 have been demonstrated to facilitate transport of LCFAs into mammalian cells. Both families of proteins are conserved in *C. elegans*, which have two FATP family members (F28D1.9 and D1009.1), as well two CD36 homologues (Y49E10.20 and Y76A2B.6).

In yeast, bacteria and mammals, intracellular activation of free fatty acids to fatty acyl-CoAs is important for efficient uptake (reviewed in [39]). Activation of fatty acids to acyl-CoAs traps these nutrients within cells, and allows them to be utilized by both catabolic and anabolic pathways. *C. elegans* have at least 7 members of the long chain acyl-CoA synthetase family, roughly twice the number found in either mammals or yeast.

Fatty acid binding proteins (FABP) and acyl-CoA binding proteins (ACBP) are an additional class of proteins important in fatty acid uptake and transport. These proteins are important both for sequestration and transport of fatty acids and fatty acyl-CoAs. *C. elegans* has 9 members of the FABP family (lbp-1 to lbp-9 and EEED8.3) and 7 members of the ACBP family (acbp1, acbp-3 to acbp-7) as well as a membrane associated ACBP, named maa-1, involved in endosomal vesicle trafficking [40].

C. elegans lack direct homologues to many mammalian lipoproteins, including A-I, B-48, B-100, C-II and E. Like other oviparous organisms, including some vertebrates, fish and insects, *C. elegans* use vitellogenins as protein components for intercellular transport of lipid particles such as yolk. The *C. elegans* vitellogenin family contains 6 members (vit-1 to vit-6), some of which exhibit moderate homology to mammalian lipoprotein B-100. Yolk is produced in intestinal cells, secreted into the pseudocoelomic fluid and taken up by developing oocytes [41]. Uptake of yolk occurs in *C. elegans* in a manner similar to uptake of mammalian lipoprotein particles. The yolk receptor RME-2 is homologous to the mammalian LDL receptor, and undergoes clathrin mediated endocytosis upon yolk binding [42]. Following uptake, endocytic sorting pathways are required for normal yolk trafficking within the oocyte [42].

In addition to the yolk trafficking pathway, the *C. elegans* gene *dsc-4* is a homolog of the mammalian microsomal triglyceride transfer protein. Two additional lipoprotein-receptor-like proteins are encoded in the *C. elegans* genome. *lrp-1* is homologous to the LDL receptor family member megalin, and is implicated in uptake of sterols in *C. elegans* [43]. *lrp-2* also shares strong homology to mammalian LDL receptors, but its function has not been well characterized.

Perspectives

There is much to be learned about mechanisms that mediate uptake, transport, storage, and utilization of lipids. As in other organisms, *C. elegans* growth, development, reproduction, and survival is fundamentally tied to this organism's capacity to maintain adequate energy supplies

in a dynamic nutritional environment. As in mammals, *C. elegans* obtain fat both from their diet and *de novo* synthesis and store neutral lipids in compartmentalized subcellular deposits. It is already clear that complex inter and intracellular mechanisms orchestrate the balance between influx and efflux of fat into and from these compartments. However, key questions regarding the biophysical characteristics, subcellular biogenesis, and regulatory mechanisms that determine size and numbers of these deposits remain to be answered. Similarly, while lipids stored in these deposits are mobilized to fuel growth and development or meet energetic demands during periods of starvation, the molecular mechanisms that orchestrate mobilization of this stored fat are largely unknown.

Because of its incredible amenability to forward and reverse genetic analyses, the speed of identifying *C. elegans* fat regulatory genes has far outstripped the pace by which their mechanisms of function are being revealed. On-going efforts to determine the subcellular localization of protein reporter fusions corresponding to various fat regulatory genes is likely to reveal protein components of *C. elegans* fat storage compartments. Transgenic animals bearing such reporter-fusions allow for dynamic monitoring of fat droplets in the context of a variety of genetic and environmental perturbations. Although still quite limited in their scope, analyses in *C. elegans* are already revealing an astonishing complexity in the regulation of fat storage and utilization by genes whose predicted biochemical activities are seemingly redundant yet, in the context of intact organisms, have distinct functions. Finally, the amenability of *C. elegans* to repeated rounds of suppressor/enhancer screens offers the unique possibility of teasing out homeostatic mechanisms that operate across various tissues to coordinate energy balance in multicellular organisms.

References

1. O'Riordan VB, Burnell AM. Intermediary metabolism in the dauer larva of the nematode *Caenorhabditis elegans* - 1. glycolysis, gluconeogenesis, oxidative phosphorylation and the tricarboxylic acid cycle. *Comp. Biochem. Physiol* 1989;92B:233–238.
2. Holt SJ, Riddle DL. SAGE surveys *C. elegans* carbohydrate metabolism: evidence for an anaerobic shift in the long-lived dauer larva. *Mech Ageing Dev* 2003;124:779–800. [PubMed: 12875742]
3. Wang J, Kim SK. Global analysis of dauer gene expression in *Caenorhabditis elegans*. *Development* 2003;130:1621–1634. [PubMed: 12620986]
4. Van Gilst MR, Hadjivassiliou H, Jolly A, Yamamoto KR. Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. *PLoS Biol* 2005;3:e53. [PubMed: 15719061]
5. McKay RM, McKay JP, Avery L, Graff JM. *C. elegans*: a model for exploring the genetics of fat storage. *Dev Cell* 2003;4:131–142. [PubMed: 12530969]
6. Ashrafi, K. WormBook. 2007. Obesity and the regulation of fat metabolism; p. 1-20.
7. Srinivasan S, Sadegh L, Elle IC, Christensen AGL, Faergeman NJ, Ashrafi K. Serotonin regulates *C. elegans* fat and feeding through independent molecular mechanisms. *Cell Metabolism*. 2008In Press
8. Kurzchalia TV, Ward S. Why do worms need cholesterol? *Nat Cell Biol* 2003;5:684–688. [PubMed: 12894170]
9. Watts JL, Browse J. Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2002;99:5854–5859. [PubMed: 11972048]
10. Suh JM, Zeve D, McKay R, Seo J, Salo Z, Li R, Wang M, Graff JM. Adipose is a conserved dosage-sensitive antiobesity gene. *Cell Metab* 2007;6:195–207. [PubMed: 17767906]
11. Yang F, Vought BW, Satterlee JS, Walker AK, Jim Sun ZY, Watts JL, DeBeaumont R, Saito RM, Hyberts SG, Yang S, Macol C, Iyer L, Tjian R, van den Heuvel S, Hart AC, Wagner G, Naar AM. An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. *Nature* 2006;442:700–704. [PubMed: 16799563]
12. Schlegel A, Stainier DY. Lessons from "lower" organisms: what worms, flies, and zebrafish can teach us about human energy metabolism. *PLoS Genet* 2007;3:e199. [PubMed: 18081423]
13. Hu, PJ. WormBook. 2007. Dauer; p. 1-19.

14. McGhee JD, Sleumer MC, Bilenky M, Wong K, McKay SJ, Goszczynski B, Tian H, Krich ND, Khattri J, Holt RA, Baillie DL, Kohara Y, Marra MA, Jones SJ, Moerman DG, Robertson AG. The ELT-2 GATA-factor and the global regulation of transcription in the *C. elegans* intestine. *Dev Biol* 2007;302:627–645. [PubMed: 17113066]
15. Tcherépanova I, Bhattacharyya L, Rubin CS, Freedman JH. Aspartic proteases from the nematode *Caenorhabditis elegans*. Structural organization and developmental and cell-specific expression of asp-1. *J Biol Chem* 2000;275:26359–26369. [PubMed: 10854422]
16. Whitcomb DC, Lowe ME. Human pancreatic digestive enzymes. *Dig Dis Sci* 2007;52:1–17. [PubMed: 17205399]
17. Hall, D. www.wormimage.org.
18. Kniazeva M, Crawford QT, Seiber M, Wang CY, Han M. Monomethyl branched-chain fatty acids play an essential role in *Caenorhabditis elegans* development. *PLoS Biol* 2004;2:E257. [PubMed: 15340492]
19. Ashrafi K, Chang FY, Watts JL, Fraser AG, Kamath RS, Ahringer J, Ruvkun G. Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* 2003;421:268–272. [PubMed: 12529643]
20. Brock TJ, Browse J, Watts JL. Fatty acid desaturation and the regulation of adiposity in *Caenorhabditis elegans*. *Genetics* 2007;176:865–875. [PubMed: 17435249]
21. Tanaka T, Ikita K, Ashida T, Motoyama Y, Yamaguchi Y, Satouchi K. Effects of growth temperature on the fatty acid composition of the free-living nematode *Caenorhabditis elegans*. *Lipids* 1996;31:1173–1178. [PubMed: 8934450]
22. Mak HY, Ruvkun G. Intercellular signaling of reproductive development by the *C. elegans* DAF-9 cytochrome P450. *Development* 2004;131:1777–1786. [PubMed: 15084462]
23. Mukhopadhyay A, Deplancke B, Walhout AJ, Tissenbaum HA. *C. elegans* tubby regulates life span and fat storage by two independent mechanisms. *Cell Metab* 2005;2:35–42. [PubMed: 16054097]
24. Hellerer T, Axang C, Brackmann C, Hillertz P, Pilon M, Enejder A. Monitoring of lipid storage in *Caenorhabditis elegans* using coherent anti-Stokes Raman scattering (CARS) microscopy. *Proc Natl Acad Sci U S A* 2007;104:14658–14663. [PubMed: 17804796]
25. Schroeder LK, Kremer S, Kramer MJ, Currie E, Kwan E, Watts JL, Lawrenson AL, Hermann GJ. Function of the *Caenorhabditis elegans* ABC transporter PGP-2 in the biogenesis of a lysosome-related fat storage organelle. *Mol Biol Cell* 2007;18:995–1008. [PubMed: 17202409]
26. Clokey GV, Jacobson LA. The autofluorescent "lipofuscin granules" in the intestinal cells of *Caenorhabditis elegans* are secondary lysosomes. *Mech Ageing Dev* 1986;35:79–94. [PubMed: 3736133]
27. Weaver TE, Na CL, Stahlman M. Biogenesis of lamellar bodies, lysosome-related organelles involved in storage and secretion of pulmonary surfactant. *Semin Cell Dev Biol* 2002;13:263–270. [PubMed: 12243725]
28. Wadsworth WG, Riddle DL. Developmental regulation of energy metabolism in *Caenorhabditis elegans*. *Dev Biol* 1989;132:167–173. [PubMed: 2917691]
29. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 1997;277:942–946. [PubMed: 9252323]
30. Van Gilst MR, Hadjivassiliou H, Yamamoto KR. A *Caenorhabditis elegans* nutrient response system partially dependent on nuclear receptor NHR-49. *Proc Natl Acad Sci U S A* 2005;102:13496–13501. [PubMed: 16157872]
31. Taubert S, Van Gilst MR, Hansen M, Yamamoto KR. A Mediator subunit, MDT-15, integrates regulation of fatty acid metabolism by NHR-49-dependent and -independent pathways in *C. elegans*. *Genes Dev* 2006;20:1137–1149. [PubMed: 16651656]
32. Costet P, Legendre C, More J, Edgar A, Galtier P, Pineau T. Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J Biol Chem* 1998;273:29577–29585. [PubMed: 9792666]
33. Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, Evans RM. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell* 2003;113:159–170. [PubMed: 12705865]

34. Desvergne B, Michalik L, Wahli W. Transcriptional regulation of metabolism. *Physiol Rev* 2006;86:465–514. [PubMed: 16601267]
35. Villena JA, Roy S, Sarkadi-Nagy E, Kim KH, Sul HS. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. *J Biol Chem* 2004;279:47066–47075. [PubMed: 15337759]
36. Narbonne P, Roy R. *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. *Nature*. 2008
37. Wang MC, O'Rourke EJ, Ruvkun G. Fat metabolism links germline stem cells and longevity in *C. elegans*. *Science* 2008;322:957–960. [PubMed: 18988854]
38. Stump DD, Fan X, Berk PD. Oleic acid uptake and binding by rat adipocytes define dual pathways for cellular fatty acid uptake. *J Lipid Res* 2001;42:509–520. [PubMed: 11290822]
39. Mashek DG, Coleman RA. Cellular fatty acid uptake: the contribution of metabolism. *Curr Opin Lipidol* 2006;17:274–278. [PubMed: 16680032]
40. Larsen MK, Tuck S, Faergeman NJ, Knudsen J. MAA-1, a novel acyl-CoA-binding protein involved in endosomal vesicle transport in *Caenorhabditis elegans*. *Mol Biol Cell* 2006;17:4318–4329. [PubMed: 16870706]
41. Kimble J, Sharrock WJ. Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. *Dev Biol* 1983;96:189–196. [PubMed: 6825952]
42. Grant B, Hirsh D. Receptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte. *Mol Biol Cell* 1999;10:4311–4326. [PubMed: 10588660]
43. Yochem J, Tuck S, Greenwald I, Han M. A gp330/megalin-related protein is required in the major epidermis of *Caenorhabditis elegans* for completion of molting. *Development* 1999;126:597–606. [PubMed: 9876188]

Figure 1. Visualization of fat depots using dye staining techniques

Nile Red staining of intestinal fat in an adult *C. elegans* with corresponding DIC image (A–B). C-12 BODIPY staining of intestinal and epidermal fat in an adult *C. elegans* with corresponding DIC (C–D). Arrows indicate epidermal lipid staining. Sudan Black fat staining (E).

Table 1

Notable differences between mechanisms of fat storage in *C. elegans* and mammals.

Mammals	<i>C. elegans</i>
Adipocytes serve as dedicated fat storage cells	Fat is stored primarily in intestinal and epidermal cells, however, some genes implicated in adipogenesis are required for fat storage capacity in <i>C. elegans</i>
Lipid droplets are contained within a phospholipid monolayer	In intestinal cells, fats are stored in lysosomal related organelle containing a phospholipid bilayer The nature of lipid storage organelle in other tissues is unknown
Perilipin, ADRP and TIP47 are lipid droplets associated proteins	No obvious sequence homologs of perilipin, ADRP and TIP47, however, homologs of other lipid droplet associated proteins such as seipin and leipin are present
Lipoproteins A-I, B-48, B-100, C-II and E are central to intercellular fat transport	Lipids are transported intercellularly via yolk, containing vitellogenins distantly related to mammalian lipoproteins
Capable of synthesizing cholesterol	Cholesterol auxotrophs
Require dietary supply of essential fatty acids 18:2n6 and 18:3n3	Capable of producing these fatty acids endogenously
Leptin and its receptor are critical for maintenance of fat storage homeostasis	No clear homologues of either leptin or the leptin receptor