Obesity and the regulation of fat metabolism*

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Abstract

As in all living organisms, survival in *C. elegans* requires adequate management of energy supplies. Genetic screens have revealed that *C. elegans* fat regulation involves a complex network of genes with known or likely functions in food sensation, neuroendocrine signaling, uptake, transport, storage and utilization of

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fats. Core fat and sugar metabolic pathways are conserved in *C. elegans.* Flux through these pathways is modulated by cellular energy sensors that operate via transcriptional and translational regulatory mechanisms. In turn, neuroendocrine mechanisms couple sensory and metabolic pathways while neuromodulatory pathways influence both metabolic and food seeking/consumption pathways. The shared ancestry of *C. elegans* and mammalian fat regulatory pathways extends to developmental programs that underlie fat storage capacity, despite lack of dedicated adipocytes, and genes whose human homologs are implicated in obesity. This suggests that many of the newly identified *C. elegans* fat regulatory pathways play similar roles in mammals. *C. elegans* is ideally suited for the integrated study of mechanisms that operate in multiple tissues and elicit feedback responses that affect processes as diverse as metabolism and behavior.

1. Obesity: an overview

Obesity is a significant risk factor for major diseases including Type II diabetes, coronary heart disease, hypertension and certain forms of cancer (Barsh et al., 2000; Kopelman, 2000; Luchsinger, 2006). Obesity arises when energy intake, principally stored as triglycerides, exceeds energy expenditure (Flier, 2004; Spiegelman and Flier, 2001). Obesity is a complex trait influenced by diet, developmental stage, age, physical activity and genes (Brockmann and Bevova, 2002; Friedman, 2003).

Genetic predisposition is a key contributing factor in obesity as demonstrated by familial aggregation, twin and adoption studies (Allison et al., 1996; Friedman, 2003; Stunkard et al., 1990). Estimates for the genetic basis of phenotypic variations in obesity range from approximately 40 to 70%. This matches or exceeds the accepted genetic contribution to height (Friedman, 2003). The idea that genetic loci alter body fat content has been substantiated by identification of mutations that cause low- or high-fat phenotypes in rodents and humans (Brockmann and Bevova, 2002; Delrue and Michaud, 2004).

There is convincing experimental evidence showing that the balance between energy intake (food consumption) and energy expenditure (basal metabolic rate, i.e. biochemical processes required to maintain cellular viability, physical activity and adaptive thermogenesis) is tightly regulated. A homeostatic network maintains energy stores through a complex interplay between the feeding regulatory centers in the central nervous system (CNS), particularly in the hypothalamus and the regulated storage and mobilization of fat stores (see Figure 1; Cone, 2005; Friedman, 2000, Flier, 2004; Sainsbury et al., 2002; Spiegelman and Flier, 2001). Thus, genes that encode the molecular components of this system may underlie obesity and related disorders.

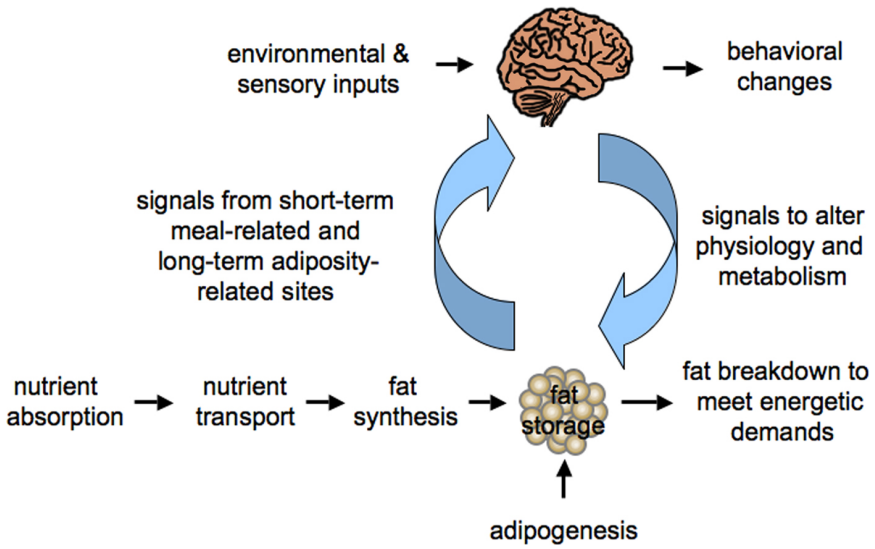

Figure 1. Homeostatic regulation of energy balance in mammals. Signals from sites of fat storage communicate the energetic state of the body to the nervous system, which also receives environmental and sensory inputs. The nervous system integrates these signals and responds to alter behavior, physiology and energy uptake, storage and utilization.

In mammals, white adipose tissue functions as the main depot for fuel storage. In the past decade, identification of myriad lipid and protein signals secreted from this tissue has led to its recognition as a major

endocrine organ (Rondinone, 2006; Trayhurn and Bing, 2006). This was principally based on the discovery of leptin, a cytokine-like hormone secreted from white adipose tissue in proportion to fat mass (Friedman and Halaas, 1998). Activation of hypothalamic leptin receptors suppresses food intake and promotes energy expenditure pathways (Friedman, 2002; Porte et al., 2002).

Insulin is another key afferent signal to the CNS that controls energy balance. Insulin is secreted from the endocrine pancreas in proportion to fat mass and exerts potent effects on peripheral nutrient storage. Similar to leptin, insulin causes long-term inhibitory effects on energy intake. There is cross talk between insulin and leptin signaling in a common set of hypothalamic neurons. Moreover, a series of neuropeptides (e.g., the melanocortin system, neuropeptide Y) and neurotransmitters (e.g., serotonin, dopamine and noradrenaline) function in the hypothalamus to coordinate behavioral, physiological and metabolic responses. Together, these responses maintain energy balance via both intake and expenditure pathways (Cone, 2005; Porte et al., 2002; Sainsbury et al., 2002).

In addition to these long-term adiposity signals, short-term meal-related signals are transmitted to the CNS through afferent nerves or gut-secreted peptides (e.g., cholecystokinin, ghrelin; Badman and Flier, 2005). Finally, neurons in the CNS also directly sense carbohydrate and fats (Demuro and Obici, 2006; Lam et al., 2005).

Because energy balance involves this complex interplay between multiple tissues and signaling pathways, an integrated view of feeding behavior, neuroendocrine signaling, nutrient uptake, transport, storage and utilization is required for understanding fat regulation. Moreover, developmental programs that underlie fat storage capacity are fundamental to understanding fat regulation. A wealth of genetic and behavioral tools makes *C. elegans* an excellent system for unraveling these complex pathways. Thus, in the past few years, the study of fat in *C. elegans* has emerged as an exciting field that is yielding new insights in the regulation of energy balance at the level of the whole organism.

2. *C. elegans* fat

2.1. Fat composition

Several groups have biochemically determined the composition of *C. elegans* fat content. This is accomplished by extraction of total lipids from whole animals, fractionation to phospholipids and neutral lipid moieties, and further analysis by column and thin-layer chromatography as well as gas-chromatography/mass spectrometry (GC/MS; Kniazeva et al., 2003; Satouchi et al., 1993; Watts and Browse, 2002). Triacylglyceride fat stores make up approximately 40–55% of total lipids depending on diet and growth stage (Ashrafi, 2006). Phospholipids pools are composed of approximately 55% ethanolamine glycerophospholipid, 32% choline glycerophospholipid, 8% sphingomyelin. Cardiolipin, inositol glycerophospholipids and lyso-cholineglycero-phospholipids account for the remaining 5%. Relative abundance of these phospholipid constituents changes with growth temperature (Satouchi et al., 1993; Tanaka et al., 1996).

Similar to mammals, *C. elegans* contains a wide range of saturated, monounsaturated and polyunsaturated fatty acids (PUFAs) including arachidonic (20:4n-6) and eicosapentaenoic acid (20:5n-3) as well as monomethyl branched chain fatty acids (mmBCFAs; Kniazeva et al., 2004; Satouchi et al., 1993; Watts and Browse, 2002). *C. elegans* combines the full range of desaturase and PUFA elongase activities that are found separately in plants and animals, abrogating the requirement for dietary supply of linoleic acid (18:2n6) and linolenic acid (18:3n3), which are essential fatty acids in mammalian diets (Brock et al., 2006; Wallis et al., 2002; Watts and Browse, 2002). Inactivation of *C. elegans* desaturase and elongase family members, encoded by *fat* and *elo* genes, respectively, causes imbalances in fatty acid composition and is associated with metabolic, physiological and behavioral phenotypes. These include altered total fat levels, growth retardation, slowed movement, reduction in body size, germ cell maintenance and reproductive defects, aberrations in rhythmic behavior, defects in sensory signaling, defects in neurotransmission and reduced adult lifespan (Brock et al., 2006; Kahn-Kirby et al., 2004; Kniazeva et al., 2004; Kniazeva et al., 2003; Lesa et al., 2003; Van Gilst et al., 2005; Watts and Browse, 2006; Watts et al., 2003). Some of the reported phenotypes may be indirect consequences of global alterations in fat levels and membrane composition.

Finally, *C. elegans* are cholesterol auxotrophs requiring dietary supply of this sterol. Given the small quantities of cholesterol needed for viability, it has been postulated that cholesterol functions as a precursor for sterol-derived hormones rather than playing a structural role in membrane composition and fluidity (Kurzchalia and Ward, 2003).

2.2. Visualization of fat droplets

Whereas mammals have dedicated adipocytes, *C. elegans* store fat in droplets in their intestinal cells and in their hypodermal cells (see Figure 2). Because *C. elegans* have transparent bodies, these fat stores can be directly visualized in intact animals. A classic method is to stain fixed animals with the fat-soluble dye Sudan Black B (see Figure 2F and G; Kimura et al., 1997), which produces a blue-black stain visible under a standard dissection microscope.

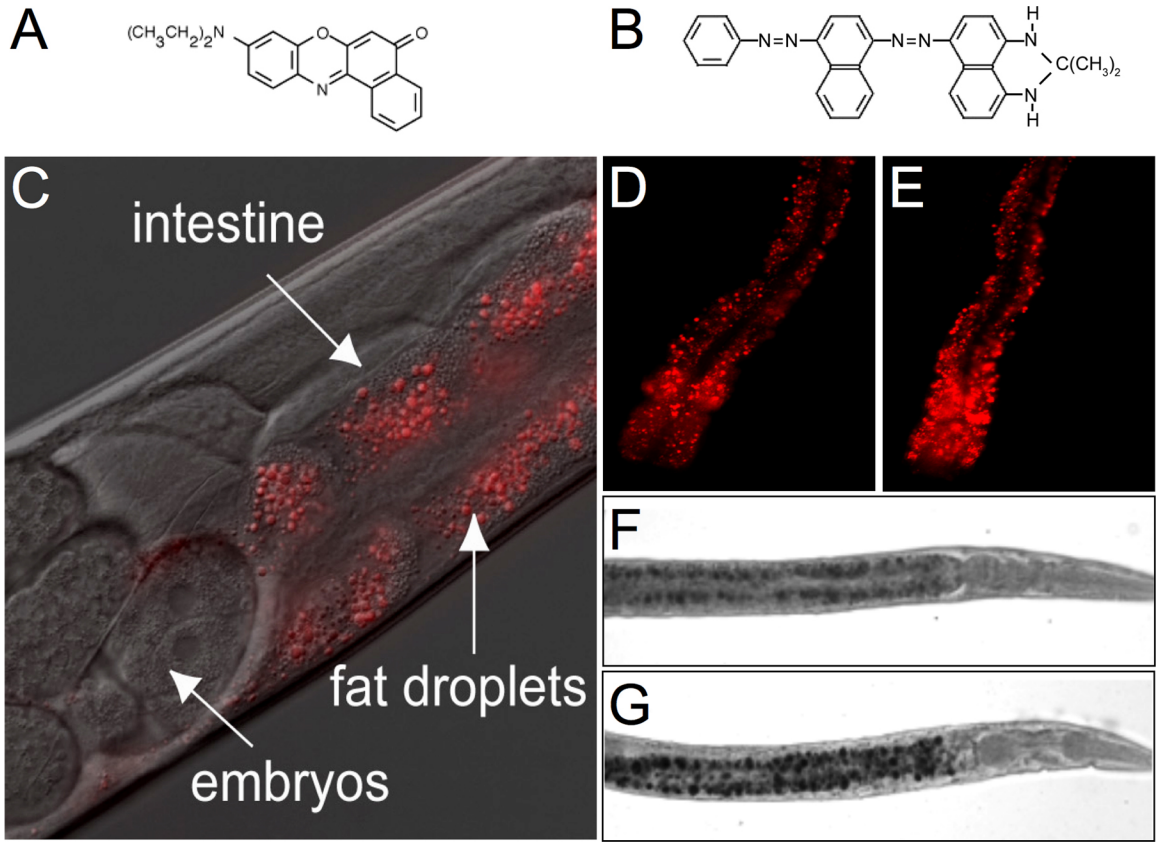

Figure 2. Visualization of intestinal lipid droplets in transparent bodies of *C. elegans*. (A-B) Chemical structures of Nile Red (A) and Sudan Black (B). (C-E) Nile Red staining in wild-type N2 animals (C, D) and *tub-1(nr2004)* mutants (E). In panels D and E, the head of the animal is toward the bottom of the panel. (F-G) Sudan Black staining in wild-type N2 animals (F) and TGF-β receptor *daf-1 (m40)* mutants. The head of the animal is to the right.

Intestinal lipid droplets can also be visualized by supplementing the normal laboratory diet of *C. elegans* with Nile Red or BODIPY-labeled fatty acids (4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid and 4,4-difluoro-5-octoyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid; Figure 2C–E; Ashrafi et al., 2003). These dyes have greatly facilitated the genetic analysis of fat regulation as they allow for easy visualization of lipids in living nematodes. These compounds are brightly fluorescent and, have been used to visualize lipid droplets in cultured mammalian cells. These vital dyes do not produce any adverse effects on *C. elegans* growth rate, brood-size, pharyngeal pumping, dauer (a larval hibernation state) formation and recovery or lifespan. One limitation is that Nile Red and BODIPY-labeled fatty acid staining methods do not distinguish between animals with reduced fat levels and animals that fail to uptake these compounds. In such cases, Sudan Black B staining is the preferred method.

Fat visualization as well as direct examination of fat composition by GC/MS have formed the basis of genetic screens for identifying *C. elegans* fat regulatory pathways (Ashrafi et al., 2003; McKay et al., 2003; Watts and Browse, 2002). Unlike the GC/MS method, Sudan Black B., Nile Red and BODIPY-labeled fatty acids do not distinguish between different lipid compositions as these dyes have a general preference for hydrophobic moieties.

2.3. Genetic analysis of *C. elegans* fat regulation

Targeted gene deletions, mutagenesis screens and a genome-scale RNA interference (RNAi) screen have identified approximately 300 gene inactivations that cause fat reduction and approximately 100 gene inactivations that cause fat accumulation without significant effects on growth and viability (Ashrafi et al., 2003; Jia et al., 2004; Kniazeva et al., 2004; Kniazeva et al., 2003; Ludewig et al., 2004; Mak et al., 2006; McKay et al., 2003; Mukhopadhyay et al., 2005; Taubert et al., 2006; Van Gilst et al., 2005; Vellai et al., 2003; Watts and Browse, 2002; Yang et al., 2006). Another approximately 250 gene inactivations cause dramatic fat reductions concomitant with defects ranging from sterility to growth arrest and lethality. Because of these pleiotropies, it is difficult to assign specific fat regulatory functions to such genes although they include some well-known components of metabolism.

The analysis of the genes identified through these screens is still in its infancy; however, recent reports demonstrate that they regulate fat content through diverse physiological processes. The shared ancestry of the mammalian and *C. elegans* fat regulatory pathways is highlighted in the sections below.

3. Metabolic pathways

Intricate metabolic networks tightly coordinate the flow of sugars and fats through synthesis, storage, and breakdown pathways. These pathways are summarized in Figure 3.

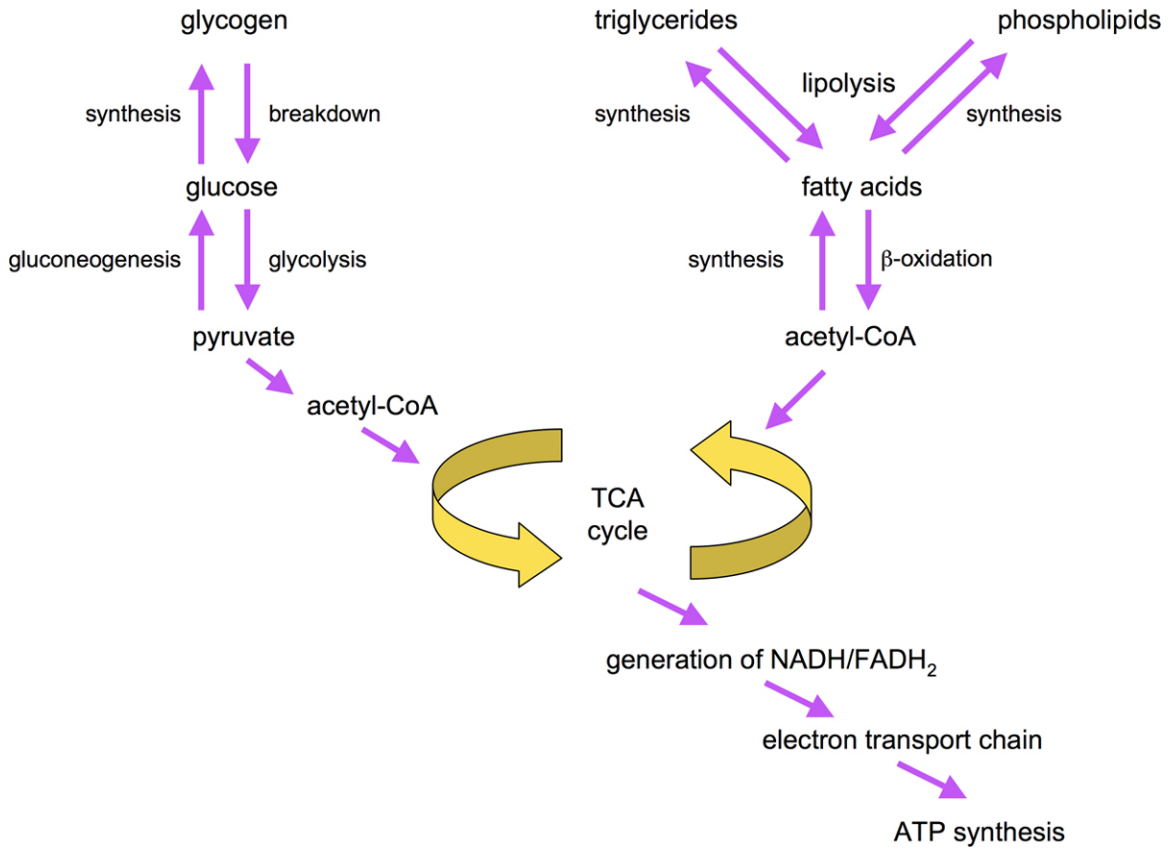

Figure 3. Overview of fat and sugar synthesis and breakdown pathways.

3.1. Breakdown pathways

In general, cells break down carbohydrates, amino acids and fats to generate ATP, the universal energy resource of cells (Salway, 2004). Carbohydrates are broken down via glycolytic enzymes to pyruvate and further to acetylCoA which powers generation of NADH and $FADH_2$ through the tricarboxylic acid cycle (TCA) cycle. In turn, NADH and $FADH_2$ are used to generate ATP via oxidative phosphorylation and ATP synthesis. Mobilization

of stored triacylglycerides is initiated by lipolytic enzymes such as hormone-sensitive lipase. Liberated fatty acids are then activated to their respective acyl-CoA derivates by acyl-CoA synthases/ligases. Breakdown of fatty acyl-CoAs to acetyl-CoA occurs in peroxisomes or mitochondria via β-oxidation enzymes (Salway, 2004; Figure 3).

3.2. Synthesis and storage pathways

Acetyl-CoA is the key substrate for synthesis of fatty acids. Acetyl-CoA is carboxylated by acetyl-CoA carboxylase (ACC) to form malonyl-CoA, which is then elongated by fatty acid synthase (FAS) in a step-wise fashion to generate fatty acids of different lengths, mainly C16:0. Products of FAS are then acted upon by fatty acid desaturases to generate unsaturated fatty acids (see Figure 3 and Figure 5). Storage of fatty acids involves the step-wise conversion of fatty acyl-CoAs derived from exogenous or endogenous sources to phosphotidic acid, diacylglycerol and ultimately triacylglycerols (see Figure 3; Salway, 2004).

Glucose is synthesized by gluconeogenic enzymes. One substrate for gluconeogenesis is glycerol, which can be derived from breakdown of triacylglycerides. Depending on tissue, excess carbohydrates may be stored as glycogen. Alternatively, they are broken down to acetyl-CoA by glycolysis, and then converted to and stored as fats. Moreover, the glyoxylate pathway allows the interconversion of carbohydrates and fats through components of the TCA cycle.

The *C. elegans* genome encodes proteins with sequence homology to conserved components of carbohydrate and lipid synthesis and breakdown. Annotations of metabolic pathways are found at KEGG and Reactome databases. A partial list is presented in Table 1.

Table 1. Partial listing of *C. elegans* metabolic pathways deduced from the genome sequence.

Carbohydrate metabolism	**Lipid metabolism**
Glycolysis/Gluconeogenesis	Lipolysis (hormone sensitive lipase)
Glycogen synthesis/Glyogen breakdown	Carnitine shuttle (fatty acid uptake)
Trehalose synthesis/Trehalose breakdown	Mitochondrial β-oxidation
Galactose metabolism	Peroxisomal β-oxidation
Fructose and mannose metabolism	Glycerol catabolism
Glyoxylate pathway	Fatty acid synthesis
Citric acid (TCA) cycle	Fatty acid elongation and desaturation
	Triacylglyceride synthesis
Energy metabolism	Phospholipids biosynthesis
Oxidative phosphorylation	Synthesis and utilization of ketone bodies
ATP synthesis	Sphingolipid and ceramide synthesis

C. elegans metabolic pathways have been examined most extensively in the context of insulin signaling. This is because down-regulation of insulin signaling confers an extended adult lifespan as well as promoting dauer formation, the larval hibernation stage (see aging and dauer chapters in Post-embryonic development section of WormBook). Increased fat accumulation and altered metabolism are hallmarks of the long-lived, stress resistant dauers. Similarly, loss of function of *daf-2*, the *C. elegans* insulin-receptor, causes fat accumulation in adults (see Figure 4). Measurements of metabolic rate, as assessed by CO_2 release, biochemical activity assessment of several key enzymes, microarray and serial analysis of gene expression, have all indicated global shifts in metabolic pathways associated with dauer larvae and *daf-2* mutant adults (Braeckman et al., 2002; Burnell et al., 2005; Gems, 1999; Halaschek-Wiener et al., 2005; Holt and Riddle, 2003; Jones et al., 2001; Larsen et al., 1995; Lee et al., 2003; Lund et al., 2002; McElwee et al., 2004; McElwee et al., 2006; Murphy et al., 2003; Van Voorhies and Ward, 1999; Wadsworth and Riddle, 1989; Wang and Kim, 2003). In general, these shifts are reminiscent of metabolic adjustments observed in nutrient deprived or fasting mammals. These adjustments favor energy conservation, fat storage, and utilization of stored reservoirs. One complexity in interpreting these studies is that they analyze mRNAs and proteins extracted from whole animals. Since different tissues play different roles in energy balance, it is likely that identical metabolic pathways are modulated differentially in separate tissues.

Figure 4. Regulation of growth and metabolism by insulin signaling in *C. elegans*. Nutrients activate signaling by the DAF-2 insulin receptor to inhibit the FOXO-transcription factor DAF-16. This promotes growth and reproduction. Nutrient limitation down regulates signaling through the insulin receptor allowing activation of DAF-16. In an early larval stage, DAF-16 activity promotes dauer formation. In adults, DAF-16 reduces reproductive rate, enhances lifespan and causes fat accumulation. The *C. elegans* genome encodes numerous insulin-like molecules and those that signal nutritional availability are not known. Activated component of insulin signaling in different contexts of nutrient availability is shown in green.

Genetic alterations of metabolic enzymes profoundly impact fat levels in *C. elegans*. For example, RNAi inactivation of key glycolic and gluconeogenic genes such as GAPDH, an insulin-regulated glycolytic enzyme, and PEPCK, a regulated enzyme of gluconeogenesis and glyceroneogenesis, cause fat reduction (Ashrafi et al., 2003). Similarly, inactivations of fatty acid synthesis (e.g., acetylCoA carboxylate and fatty acid synthase), phospholipid synthesis (e.g., serine palmitoyltransferase, choline/ethanoloaminephosphotransferase) and triglyceride synthesis (e.g., glycerol-3-phosphate acyltransferase) pathways cause reductions in lipid accumulation and, in some cases, are associated with growth arrest (see Figure 3 and Figure 5; Ashrafi et al., 2003). Inactivation of fatty acid oxidation genes causes either decreased or increased fat levels (Ashrafi et al., 2003; Mak et al., 2006; Van Gilst et al., 2005). The basis for this paradoxical result is not yet clear but likely reflects compensatory and homeostatic mechanisms. Not surprisingly, inactivation of oxidative phosphorylation and ATP synthesis components is generally associated with profound reductions in fat levels concomitant with growth defects (K. Ashrafi, unpublished observations).

Inhibition of *fat-5*, *fat-6*, and *fat-7* genes encoding delta-9 fatty acid desaturation enzymes is associated with reduced fat levels. Interestingly, RNAi inactivation of *fat-7* causes fat reduction and shortened lifespan, phenotypes not seen in a *fat-7* deletion mutation (Brock et al., 2006; Van Gilst et al., 2005). This discrepancy may be explained by the observation that loss of function mutations in *fat-6* or *fat-7* cause compensatory transcriptional responses in the remaining delta-9 desaturase genes. Accordingly, triple *fat-5; fat-6; fat-7* are embryonic lethal (Brock et al., 2006).

Mammalian delta-9 stearoyl-CoA desaturase-1 (SCD-1) has emerged as a therapeutic target for obesity and metabolic disorders. SCD-1 is a target of leptin signaling. Significantly, SCD-1 knock-out mice display dramatic reductions in adiposity on otherwise wild-type or leptin deficient (*ob/ob*) backgrounds (Cohen and Friedman, 2004; Cohen et al., 2003). SCD-1 deficiency promotes β-oxidation pathways and decreases lipogenesis in liver and skeletal muscle. One proposed mechanism is that SCD-1 inhibition results in accumulation of saturated fatty acylCoAs which cause feedback inhibition of acyl-CoA carboxylase (ACC), the rate-limiting enzyme of fatty acid synthesis. ACC inhibition results in reduced accumulation of its product, malonylCoA, which in turn, relieves inhibition of carnitine-palmityol-transferase (CPT) shuttle. This allows for transport of fats into mitochondria for breakdown via β-oxidation (see Figure 5; Cohen and Friedman, 2004; Cohen et al., 2003). Whether similar mechanisms account for fat reduction of *C. elegans* deficient in desaturase activity is not known. However, as in mammals, *C. elegans* delta-9 fatty acid desaturases are transcriptionally regulated by sterol response element binding protein and nuclear hormone receptors (see sections 4.1 and 4.2 below; Brock et al., 2006; Taubert et al., 2006; Van Gilst et al., 2005)

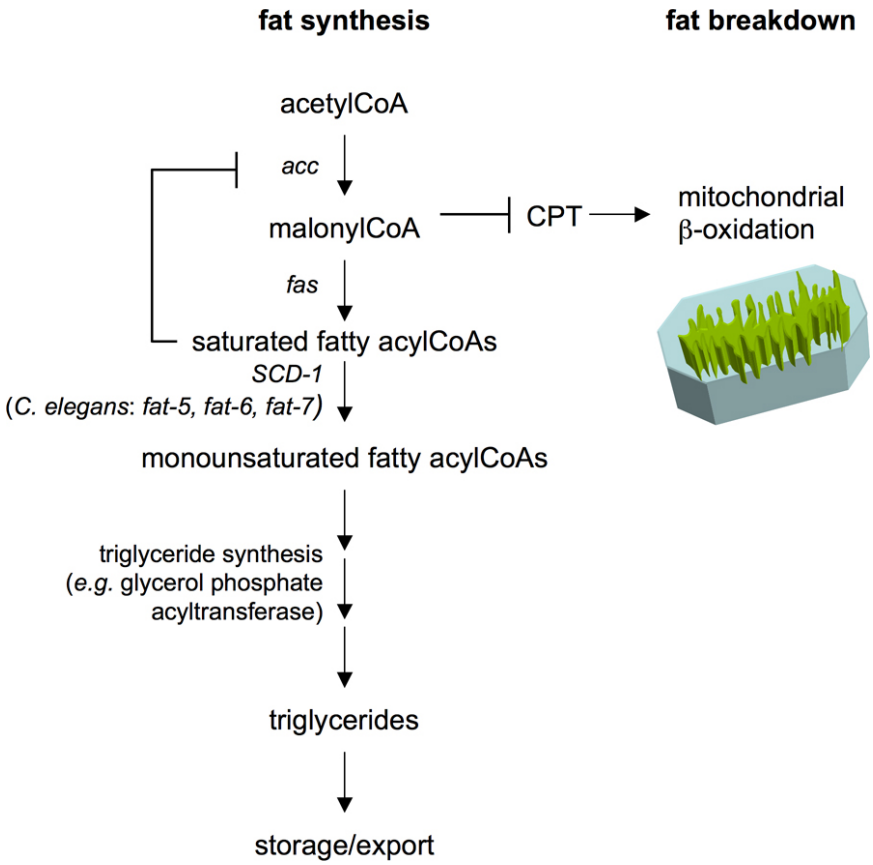

Figure 5. Coordination of fat synthesis and breakdown pathways by malonyl-CoA. AcetylCoA is the building block of fatty acids that are assembled into storage triglycerides through step-wise enzymatic processes. Inhibiting delta-9 desaturase activity (*SCD-1* in mammals, *fat-5*, *fat-6*, *and fat-7* in *C. elegans*) causes accumulation of saturated fatty acids. Consequently, acetylCoA carboxylase (ACC) is inactivated through feedback inhibition. This results in reduced levels of the ACC product malonyl-CoA. Since malonyl-CoA is an inhibitor of carnitine-palmitoyl-transferase (CPT), ACC inhibition results in activation of CPT, allowing uptake of fatty acids into mitochondria where they are broken down via β-oxidation. FAS: fatty acid synthase.

4. Metabolic sensors and coordinated regulation of metabolic pathways

The capacity to coordinately adjust energy flux through various catabolic and anabolic pathways in response to changing nutritional status is critical for cellular and organismal survival. Metabolic sensing mechanisms are thought to coordinate these responses (Lindsley and Rutter, 2004). How alterations in energy status are sensed is a vibrant field of research. On a cellular level, metabolic sensors respond to altered concentrations of macronutrients, e.g., glucose, amino acids and fatty acids, metabolites derived from these macronutrients and energy resources such as ATP and NADH. In multicellular organisms, energetic status of different tissues is further coordinated through hormonal signals (Lindsley and Rutter, 2004; Salway, 2004). Recent studies in mammals indicate that some of these cellular metabolic sensors also function in the nervous system to regulate behavioral responses (Minokoshi et al., 2004; Porte et al., 2005). Several such *C. elegans* pathways are highlighted below:

4.1. *sbp-1*

Sterol response element binding protein (SREBP) is a key transcriptional regulator of fat and sterol synthesis pathways in mammals (Eberle et al., 2004; Rawson, 2003). RNAi inhibition and loss of function mutations in *C. elegans* SREBP, Y47D38.7/*sbp-1/lpd-1*, cause dramatic reductions in fat content and biogenesis of intestinal lipid droplets (Ashrafi, 2006; McKay et al., 2003; Yang et al., 2006). Thus far, analysis of candidate *sbp-1* targets in *C. elegans* has been reported for malic enzyme (ME), and ATP citrate-lyase (ACL), acyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturases (FAT-6/FAT-7), and glycerol 3-phosphate acyltransferase (G3PA; Figure 5; McKay et al., 2003; Yang et al., 2006). The mammalian homologs of each of these lipogenic genes are direct SREBP targets. Additionally, *sbp-1* regulates expression of *elo-5* and *elo-6*, two fatty acid elongation enzymes required for synthesis of monomethyl branched chain fatty acids (Kniazeva et al., 2004).

Further conservation of function for *sbp-1* has emerged from studies in which *sbp-1* stimulated transcription of mammalian SREBP targets in a human cell line. Moreover, in both mammalian cells and *C. elegans*, SREBP function requires physical interaction with a transcriptional co-activator, ARC105/MDT-15 (Yang et al., 2006; see section 4.2).

Given that *C. elegans* are cholesterol auxotrophs, it remains to be determined whether any sterol metabolic pathways are regulated by *sbp-1*. One function of cholesterol in mammalian cells is to regulate membrane fluidity. Interestingly, *Drosophila melanogaster*, also a cholesterol auxotroph, regulates its membrane fluidity through an SREBP-mediated transcriptional program that produces phosphotidylethanolamine (Seegmiller et al., 2002). It is likely that a similar mechanism regulates membrane fluidity in *C. elegans.* Also, it remains to be determined if, as in mammals, *C. elegans* insulin signaling regulates *sbp-1* function.

4.2. *nhr-49/mdt-15*

In mammals, several nuclear hormone receptors (NHRs) function as metabolic sensors and master regulators of energy balance (Chawla et al., 2001; Evans et al., 2004; Nakamura et al., 2004). The *C. elegans* genome contains a remarkable number of NHRs yet none display sequence homology to the Peroxisome Proliferator-Activated Receptor (PPAR) family, which are key regulators of fat, cholesterol and glucose homeostasis (Gissendanner et al., 2004; Sluder and Maina, 2001). Instead, the *C. elegans* NHR-49/MDT-15 system serves a parallel function to mammalian PPARα and its co-activator, PGC-1.

A deletion mutation in the HNF-4α family member *nhr-49* mimics the high fat phenotype of *nhr-49* RNAi (Ashrafi et al., 2003; Van Gilst et al., 2005). Analysis of genes in fat metabolic pathways (Table 1) by quantitative RT-PCR revealed that *nhr-49* causes down-regulation of three genes encoding mitochondrial β-oxidation enzymes and concomitant up-regulation of three genes that encode peroxisomal β-oxidation enzymes (Van Gilst et al., 2005). Down-regulation of *acs-2* and *ech-1*, two of the three affected mitochondrial β-oxidation genes, causes fat accumulation, and over-expression of *acs-2* suppresses the high fat phenotype of *nhr-49* (Van Gilst et al., 2005). This suggests that down-regulation of mitochondrial β-oxidation underlies excess fat levels of *nhr-49* inactivation. Similar to mammalian PPARα *nhr-49* also regulates expression of fatty acid desaturation and lipid binding proteins. Interestingly, changes in expression patterns of fat metabolic genes caused by *nhr-49* inactivation overlap with expression changes noted after 12 hours of food deprivation (Van Gilst et al., 2005; Van Gilst et al., 2005). Thus, *nhr-49* responds to nutrient signals and functions as a regulatory node of metabolic gene expression.

The transcriptional co-activator, *mdt-15/arc105*, was found to interact with *nhr-49* in a yeast two-hybrid assay (Taubert et al., 2006). Many of the metabolic gene expression changes noted for *nhr-49* are similarly altered in *mdt-15/arc105* knock-down animals. Although overlapping, the full complement of expression changes caused by *mdt-15/arc105* and *nhr-49* are not identical, raising the possibility that *mdt-15/arc105* functions as a co-activator for other transcription factors. Indeed, several other NHRs bind MDT-15/ARC105 in yeast two-hybrid experiments (Taubert et al., 2006). Interestingly, MDT15/ARC105 was recently characterized as a co-activator of SBP-1/SREBP in *C. elegans* and mammals (Yang et al., 2006). *mdt-15/arc105*, *sbp-1*, *nhr-49*, and *nhr-80* control expression of delta-9 fatty acid desaturase genes (*fat-5*, *fat-6*, and *fat-7*) illustrating complex regulatory mechanisms of fat metabolic pathways (Brock et al., 2006; Taubert et al., 2006; Van Gilst et al., 2005).

Several other NHRs, whose mechanisms of function are unknown, are also required for wild type intestinal fat deposits (Ashrafi et al., 2003). Finally, another NHR, *daf-12*, and its co-factor *din-1* are components of a steroid-based hormonal signaling pathway that controls entry into the high fat dauer state; however, the relationship of the DAF-12/DIN-1 complex to metabolic pathways is not yet clear (Antebi et al., 2000; Ludewig et al., 2004).

4.3. TOR, AMPK, and hexosamine pathways

TOR (target of rapamyacin) is an evolutionarily conserved phosphatidylinositol kinase related family member that couples cell size and proliferation to nutrient levels, particularly amino acids and hormonal signals such as insulin (Inoki and Guan, 2006; Lindsley and Rutter, 2004). In *Saccharomyces cerevisiae, Drosopholia melanogaster* and mammalian cells, TOR activity promotes translation through direct activation of translational machinery. The precise nature of the nutrient signal that elicits TOR activity remains elusive. Loss of function mutations as well as RNAi inactivation of *C. elegans* TOR (*let-363*/B0261.2) and its partner Raptor (*daf-15*/C10C5.6) cause developmental arrest and fat accumulation (Jia et al., 2004; Vellai et al., 2003). Genetic analysis places raptor/*daf-15* downstream of insulin signaling and upstream of the eIF-4G and eIF-2 subunits of translational machinery (Jia et al.,

2004). In *Drosophila melanogaster* and mammals, the TSC tumor suppressor complex provides a mechanism of cross talk between the insulin and TOR signaling pathways. The *C. elegans* genome lacks sequence identifiable TSC complex components, TSC-1 and TSC-2. Thus, despite differences in cross talk mechanisms, insulin and TOR pathways interact in *C. elegans* to couple nutrient availability to growth and metabolism (Long et al., 2004; Long et al., 2002).

The AMP-activated kinase (AMPK) is a major cellular fuel gauge as its activity is responsive to cellular AMP:ATP ratio as well as upstream kinase cascades (Kahn et al., 2005; Lindsley and Rutter, 2004). AMPK activation causes numerous cellular changes, that together down-regulate energy-consumptive pathways and up-regulate energy-generating pathways. There is extensive cross talk between insulin, AMPK and TOR signaling pathways. In mammals, neuronal AMPK also functions downstream of leptin and insulin signaling to modulate food intake (Kahn et al., 2005; Lindsley and Rutter, 2004). Thus, AMPK is a major target of therapeutic intervention for metabolic syndromes such as type II diabetes.

Genes corresponding to catalytic α and regulatory β and γ subunits of AMPK are conserved in *C. elegans* and kinase activity for one of the two catalytic α subunits has been demonstrated (Apfeld et al., 2004; Curtis et al., 2006; Narbonne and Roy, 2006). Genetic analysis has linked the *C. elegans* AMPK cascade to insulin and mitochondrial pathways. These studies have focused on adult lifespan as a read-out. Direct investigation of the effect of *C. elegans* AMPK on fat regulatory pathways has not yet been reported.

O-linked *N*-acetylglucosamine (*O*-GlcNAc) is thought to function as a dynamic posttranslational modification of many proteins (Lindsley and Rutter, 2004; Love and Hanover, 2005). Production of uridine 5′-diphospho-*N*-GlcNAc (UDP-GlcNAc), the *O*-GlcNAc donor, occurs through the hexosamine biosynthetic pathway. O-GlcNAC transferase (OGT) and O-GlcNAC glycosidase (OGA) add and remove *O*-GlcNAc from target proteins. Flux through the hexosamine pathway is tuned to cellular energy levels. This allows for global alterations in functions of *O-GlcNAC* modified target proteins. Defects in this pathway are associated with numerous diseases including Type II diabetes. Deletions in *C. elegans ogt-1 and oga-1* have been reported to increase glycogen and trehalose levels while decreasing fat levels. Moreover, insulin mediated dauer pathways are affected in *ogt-1* and *oga-1* mutant animals (Forsythe et al., 2006; Hanover et al., 2005; Love and Hanover, 2005).

5. Development of fat storage capacity

During mammalian adipogenesis, hormonal cues initiate transcriptional programs that guide the differentiation of multipotent mesenchymal stem cells into mature adipocytes. Members of bZIP CCAAT/enhancer binding protein (C/EBP) transcription factor family and PPARγ are key components of these transcriptional cascades (Rosen, 2005). SREBP is also required for lipogenic programs of differentiating adipocytes. Despite the fact that *C. elegans* intestinal cells, the major site of fat deposition, are endodermal derivatives, the *C. elegans* counterpart of C/EBP (C48E7.11) and *sbp-1* are expressed in intestinal cells and are required for fat storage (McKay et al., 2003). Electron microscopic examination of a deletion mutation of *sbp-1*, *lpd-1(gf1)*, revealed that, intestinal cells of these animals maintain overall normal ultrastructural appearance including intact microvilli (McKay et al., 2003). This suggests that fat storage capacity of intestinal cells is distinct from developmental program of these cells as enterocytes.

McKay and colleagues also found that RNAi inactivation of each of eight genes (*lpd-3* though *lpd-9* and *mac-1*) causes morphological and fat phenotypes reminiscent of *sbp-1* and C/EBP inactivation. *lpd-4* (F26E9.4) and *lpd-5* (ZK973.10) encode components of complex IV and complex I of the mitochondrial respiratory chain, respectively. Chemical inhibition of complex I and complex IV by rotenone and NaN_3 causes reduction of lipid accumulation in 3T3-L1 cells, a murine tissue culture adipocyte model system. Moreover, *lpd-3* encodes a novel but conserved gene expressed in *C. elegans* intestinal cells. The mammalian counterpart of *lpd-3* is strongly expressed in brain, testis and embryonic fat tissues. Inactivation of mammalian *lpd-3* by shRNA in 3T3-L1 cells that had been induced to undergo adipogenesis prevented lipid accumulation despite appearance of adipocyte differentiation markers (McKay et al., 2003).

6. Lipid uptake/transport

The *C. elegans* genome encodes proteins with sequence homology to fatty acid translocase (FAT/CD36), fatty acid transport protein (FATP), fatty acid binding proteins (FABPs), acyl-CoA binding proteins (ACBPs), carnitine-palmitoyl transferases (CPTs) and ATP-binding-cassette (ABC) transporter proteins. Mammalian homologs of these genes mediate fatty acid transport across various lipid bilayers and intracellular shuttling of fatty

acylCoAs. In mammals, altered expression of putative fatty acid transport proteins is associated with obesity and insulin resistant states (Chawla et al., 2001; Koonen et al., 2005; Mashek and Coleman, 2006).

Loss of function mutations and RNAi inactivation of specific members of FABP, ACBP, CPT and ABC transporters cause increased or decreased intestinal fat levels as visualized by Nile Red staining (Ashrafi et al., 2003). Loss of function of a FATP-like transporter causes a reduced fat phenotype only in the context of mutations that confer increased fat storage (K. Ashrafi, unpublished results). Additionally, RNAi inactivations of specific family members of OCT-type transporters, a lysosomal transporter, an amino-acid permease and glucose transporters cause altered fat accumulation (Ashrafi et al., 2003). Mechanisms of function underlying these phenotypes are not known.

Similarly, inactivation of the *opt-2/pep-2* and *nhx-2* transporters cause reduced fat accumulation (Ashrafi et al., 2003; Meissner et al., 2004; Nehrke, 2003). GFP-reporter fusions for each of these genes are exclusively expressed along the apical membrane of intestinal epithelia. OPT-2 is a transporter of di- and tripeptides and NHX-2 is a Na^+/H^+ exchanger. These functions are required for appropriate acidification of intestinal cells, which in turn, powers a variety of proton-coupled nutrient uptake systems.

The best characterized lipid uptake and transport system in *C. elegans* has been delivery of nutrients from intestinal cells to developing embryos (see Intracellular trafficking). A mixture of fats and cholesterol are loaded onto vitellogenins, yolk proteins with functional and structural similarities to LDL-type proteins. Vitellogenins are secreted from the intestinal cells into the pseudocoelum and then taken up by developing embryos via receptor-mediated endocytosis (Fares and Grant, 2002). Finally, a conserved, transmembrane ACBP, *maa-1*, is associated with Golgi and endosomal membranes. This ACBP modulates vesicular transport in the intestine, hypodermis and oocytes and, when inactivated, impairs receptor-mediated endocytosis (Larsen et al., 2006).

7. Neuroendocrine fat and feeding regulatory pathways

In mammals, the nervous system functions as a central coordinator of both metabolic pathways and behaviors associated with food consumption. The *C. elegans* nervous system also regulates fat storage both in conjunction with and independent of feeding pathways.

7.1. Insulin and TGF-β

Signaling cascades through insulin, transforming growth factor (TGF-β) and cyclic nucleotide regulated pathways control whether *C. elegans* larvae grow to adults or fat-storing dauers. Molecular components of these pathways are extensively covered elsewhere. Down-regulating either insulin or TGF-β pathway components promotes fat accumulation in adults. A clear example of neuronal regulation of fat levels is provided by DAF-7, a TGF-β ligand. *daf-7* is expressed in one pair of ciliated sensory neurons (ASI) and its transcription is modulated by daumone, a constitutively secreted pheromone that *C. elegans* use to assess population density (Jeong et al., 2005; Ren et al., 1996). Thus, this pathway responds to environmental conditions and function as a central regulator of *C. elegans* homeostasis.

Similarly, many of the *C. elegans* insulins are expressed in neurons and have been postulated to relay changes in food availability, although direct evidence for this is lacking (Pierce et al., 2001). In mammals, insulin signaling has both peripheral and central actions on fat homeostasis (see Figure 6). Importantly, tissue-specific knockouts or reconstitution of the insulin receptor in mice has begun to reveal contributions of different tissues to glucose and fat homeostasis. For instance, neuronal insulin receptor knockout and muscle insulin receptor knockout mice are obese while fat cell insulin receptor knockout mice are lean and resistant to diet induced obesity (Biddinger and Kahn, 2006). Similarly, insulin signaling in different *C. elegans* tissues contributes differentially to fat content. For instance, reconstitution of the insulin receptor in neurons but not in muscle partially rescues the increased fat content of insulin receptor knockout animals (Wolkow et al., 2000).

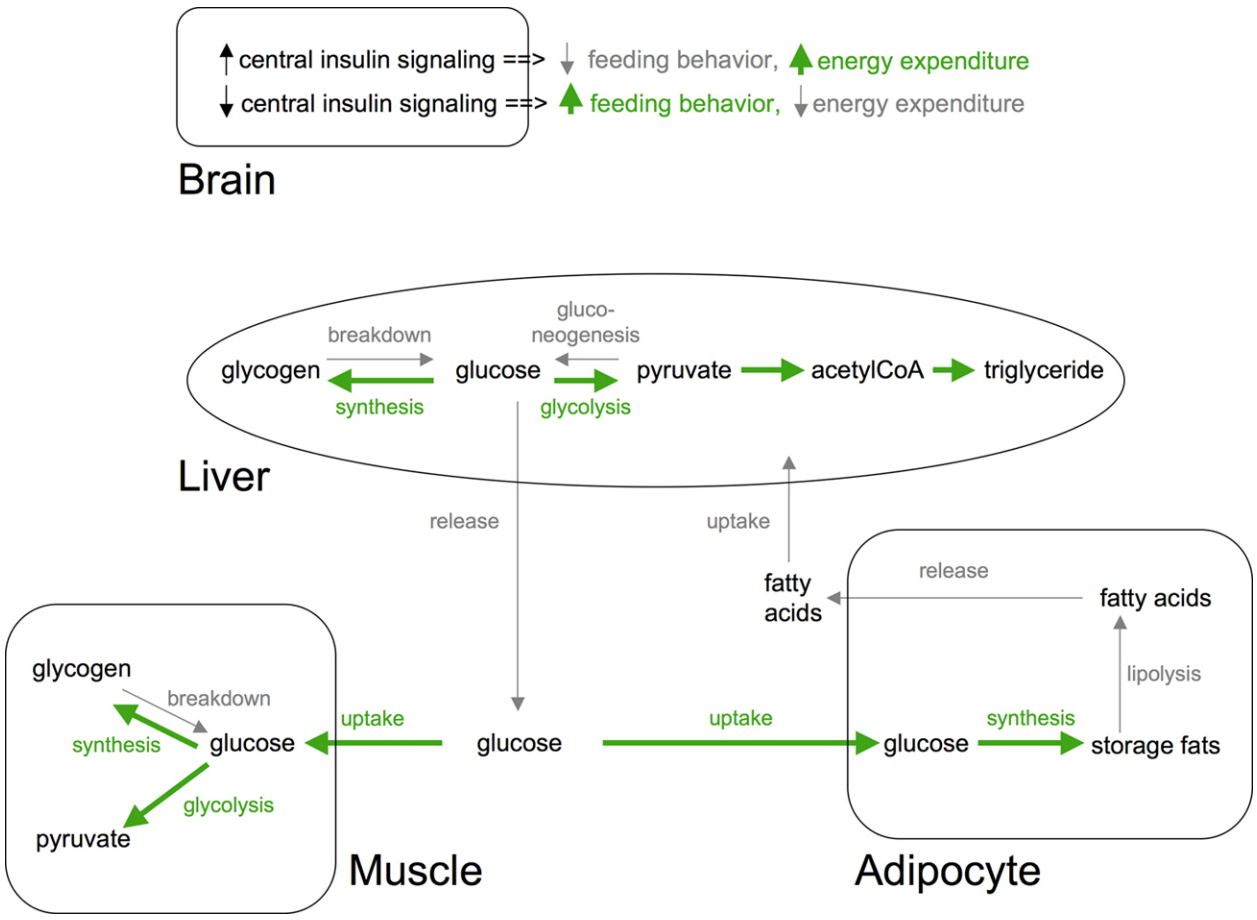

Figure 6. Systemic actions of insulin signaling in mammals. In response to nutrients (e.g., glucose) insulin signaling promotes uptake and storage of glucose as glycogen and triglycerides in muscle and adipocytes, respectively. Concomitantly, insulin inhibits triglyceride and glycogen breakdown pathways in these tissues. Similarly, insulin inhibits hepatic gluconeogenesis and glycongenolysis while promoting glycogen synthesis. In both liver and muscle, insulin also promotes glucose breakdown. Insulin signaling in brain inhibits feeding behavior and activates energy expenditure pathways. Tissue-specific insulin receptor knockouts have revealed complex compensatory mechanisms. Pathways activated by insulin signaling are shown in green. Pathways inhibited are shown in light gray. Additional actions of insulin on protein synthesis/breakdown and other tissues are not shown.

7.2. Serotonin, dopamine and glutamate pathways

Classical neurotransmitters have dramatic effects on fat regulation in nemotodes and in mammals. Deleting *tph-1*, which encodes the enzyme tryptophan hydroxylase, causes *C. elegans* to lack serotonin (Sze et al., 2000). Serotonin production is largely confined to ADF, a head sensory neuron, NSM, a pharyngeal neuron, HSN, a hermaphrodite-specific neuron that innervates the vulva, and sensory neurons innervating the male tail. Serotonin-deficient animals are viable but have excess fat accumulation, reduced feeding rate (see section 7.4) and reduced rate of progeny production (Sze et al., 2000). Genetic analysis suggests interactions between serotonin, insulin and TGF-β pathways (Sze et al., 2000).

Additionally, inactivating specific dopaminergic and glutamergic receptors alters fat deposits without adversely affecting growth rate or viability (Ashrafi et al., 2003). Mammalian counterparts of these neuromodulatory pathways have also been implicated energy balance (Clifton and Kennett, 2006; Sainsbury et al., 2002).

7.3. *tub-1* and *bbs-1*

Mutations in rodent *tubby* cause progressive degeneration in retinal and cochlear sensory receptor cells, infertility and adult-onset obesity with insulin resistance (Carroll et al., 2004). *Tubby* is broadly expressed in the central nervous system including the hypothalamus. Molecular mechanisms of *Tubby* function are unclear although several models have been proposed (Carroll et al., 2004). Loss of function in *tub-1*/F10B5.5, the *C. elegans* ortholog of *Tubby*, causes fat accumulation (Ashrafi et al., 2003; Mak et al., 2006). A functional TUB-1::GFP fusion localizes

to all ciliated sensory neurons in *C. elegans* (Mak et al., 2006; Mukhopadhyay et al., 2005). In a yeast two-hybrid assay, TUB-1 was found to interact with B0393.2, a predicted RabGTPase-activating protein (RabGAP) postulated to function in vesicular transport (Mukhopadhyay et al., 2005). This RabGAP is expressed in the amphid and phasmid subset of ciliated sensory neurons. RNAi inactivation of this RabGAP causes only a minor reduction in fat content of wild-type animals but suppresses the excess fat of *tub-1* mutant animals (Mukhopadhyay et al., 2005). This suggests a surprisingly specific role for vesicular transport in accumulation of excess fat in *tub-1* deficient animals. Moreover, *tub-1* mutant animals have extended lifespan. This lifespan extension requires insulin signaling but appears to be independent of the TUB-RabGAP fat pathway (Mukhopadhyay et al., 2005).

Neuronal *tub-1* has been reported to act synergistically in fat accumulation with *kat-1/T02G5.8*, which encodes a non-neuronal β-oxidation enzyme, 3-ketoacyl-coA thiolase (Mak et al., 2006). The synergistic nature of the excess fat accumulation in *tub-1;kat-1* double mutants suggests that defects in neuronal *tub-1* are normally compensated by *kat-1* mediated fat oxidation in non-neuronal tissues. Loss of *kat-1* abrogates this multi-tissue compensatory mechanism.

The molecular nature of compensatory mechanisms that couple *tub-1* and *kat-1* are not yet known; however, genetic analysis of *kat-1* led to identification of *bbs-1* as another modifier of intestinal fat storage that, like *tub-1*, functions in ciliated neurons (Mak et al., 2006). Mutations in human ortholog of *bbs* genes including *bbs-1* underlie Bardet-Biedl syndrome, a pleiotropic syndrome associated with obesity (Beales, 2005). Many human *BBS* genes, which are implicated in ciliogenesis and intraflagellar transport (IFT), have *C. elegans* homologs (Inglis et al., 2006). Similar to *tub-1*, loss of function mutations in *bbs-1* cause modest increases in fat accumulation that are exacerbated by loss of KAT-1 (Mak et al., 2006; Mukhopadhyay et al., 2005). Moreover, *tub-1* mutants have defects in chemotaxis, a function mediated by a subset of ciliated sensory neurons, and there is evidence that TUB-1 undergoes IFT (Mak et al., 2006; Mukhopadhyay et al., 2005; see Chemosensation). Together, these findings suggest that *tub-1* and *bbs-1* function in the same fat regulatory pathway.

The provocative hypothesis that *bbs-1* and *tub-1* form a neuroendocrine axis with *kat-1* is based on the synergistic rather than additive fat content of double mutants as assessed by Nile Red fluorescence. The potential insights offered by such genetic interactions highlight the need for standard methods to accurately quantify fluorescence intensity.

7.4. Feeding behavior and fat pathways

C. elegans feed by pumping and concentrating food using a neuromuscular organ known as the pharynx (Avery and Shtonda, 2003; Shtonda and Avery, 2005). The grinder, a teeth-like structure located at the junction of the pharynx and the intestine, breaks food particles that are then pushed into the lumen of the intestine by the peristaltic pumping action of the pharynx. *C. elegans* pump in the presence and absence of food; however, pumping rate is modulated by food availability (Avery and Horvitz, 1990). Animals that have experienced starvation will pump faster when re-exposed to food than well-fed animals. *C. elegans* also forage for food. Rates and patterns of *C. elegans* movement are different compared on or off food. These locomotory rates and patterns are also modulated by starvation (Hills et al., 2004; Sawin et al., 2000).

Serotonin modulates pumping rate. *tph-1* mutant animals display reduced pumping rate while animals exposed to excess serotonin or imipramine, a serotonin uptake inhibitor, display increased pumping (Avery and Horvitz, 1990; Horvitz et al., 1982). Pumping stimulatory effects of serotonin are abrogated by mutations in each of two serotonergic receptors *ser-1* and *ser-7* (Hobson et al., 2006). Additionally, serotonin, dopamine and glutamate signaling pathways are implicated in different foraging strategies of *C. elegans* (Hills et al., 2004; Sawin et al., 2000). These neuronal signaling mechanisms also modulate mammalian feeding behavior (Clifton and Kennett, 2006; Sainsbury et al., 2002).

Together, these results indicate an overlap between neuronal feeding and foraging behavior pathways and central fat regulatory mechanisms; however, the nature of these relationships is not yet clear. For instance, there is an inverse correlation between fat content and pumping rate for serotonin deficient animals. In other cases, such as *tub-1* mutants, animals display wild-type pumping rates despite increased fat levels.

8. Perspectives

Our understanding of body fat regulation as a homeostatic, organismal process has flourished in the past decade. Although many of the core metabolic pathways were biochemically defined long ago, integration and

coordination of these pathways across multiple tissues is a vibrant field of integrative biology. This is because understanding fat regulation requires multiple layers of investigation spanning from metabolism, transcription and signaling to neuronal development and behavior. Deciphering neuronal circuits that coordinate behavior, physiology, and metabolism is a major challenge in understanding fat regulation. Similarly, compensatory mechanisms that operate at organismal level to maintain energy homeostasis are just being elucidated.

The genetic tractability of *C. elegans* has already revealed that mechanisms of energy balance in this organism range from neuronal sensation and endocrine signals to nutrient uptake, transport and storage/utilization mechanisms. Importantly, amenability of *C. elegans* to multiple rounds of suppressor/enhancer screening is critical and provides a unique advantage for understanding homeostatic feedback regulatory mechanisms. Examining fat regulatory pathways under different environmental conditions holds the potential to reveal how physiological pathways are coordinately modulated in response to environmental perturbation. Similarly, how developmental stage, age, experience and diet perturb and possibly rewire the fat networks can be addressed in *C. elegans* at a molecular level. Finally, *C. elegans* is well suited for deciphering developmental programs that underlie fat storage capacity and cell biological determinants of lipid droplet biogenesis.

Many of the adverse health effects of excess fat accumulation in humans are unlikely to occur in *C. elegans*. Nevertheless, the limited number of studies reported thus far already reveal remarkable similarities between molecular components of mammalian and *C. elegans* fat pathways that extend to disease-associated genes. Many of the fat genes identified in *C. elegans* have mammalian homologs whose roles in energy balance have not yet been examined. Given that energy balance is fundamental for viability, it is likely that many of the newly identified *C. elegans* fat regulatory networks are functionally conserved in mammals.

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