Chapter 14
Physiological Functions and Regulation of *C. elegans* Stearoyl-CoA Desaturases

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The *Caenorhabditis elegans* Model for Studies of Lipid Synthesis and Function

The nematode *C. elegans* is an excellent model for genetic studies of complex biological phenomena, and research using this organism has contributed significantly to elucidating the mechanisms of diverse biological processes (Barr 2003). Recent work in *C. elegans* has identified many regulatory proteins and downstream effector genes responsible for lipid homeostasis (Watts 2009; Vrablik and Watts 2012). While *C. elegans* stores lipids in intestinal and hypodermal cells rather than dedicated adipose tissue, other aspects of worm biochemistry and regulation of fat metabolism closely parallel humans. Because of its small size (1.5 mm), rapid life cycle, and ease of laboratory cultivation, *C. elegans* offers great potential for genetic analysis (Riddle et al. 1997). In addition to the traditional forward genetic approaches, reverse genetic studies are popular and powerful because the activity of a target gene can be inactivated in vivo by feeding *C. elegans* bacteria producing double stranded RNA for that gene, a technique referred to as RNA interference, or RNAi (Fire et al. 1998). During feeding the double stranded RNA is absorbed by the intestine and distributed throughout the animal (Timmons and Fire 1998). The construction of RNAi libraries that consist of *E. coli* strains expressing double stranded RNA that correspond to nearly every *C. elegans* gene has allowed for whole-genome screening approaches to identify genes involved in diverse biological processes. These processes include embryogenesis (Sonnichsen et al. 2005), neuronal specification (Poole et al. 2011), and the regulation of longevity (Hamilton et al. 2005).

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C. elegans contains genes encoding enzymes required for fatty acid synthesis, elongation, and desaturation as well as those encoding peroxisomal and mitochondrial β-oxidation enzymes. Fatty acids are esterified to glycerol to form triacylglycerols (TAGs), which are stored in lipid droplets and yolk. In *C. elegans*, TAGs are a vital energy source during embryogenesis, periods of low food availability, and for the specialized non-feeding dauer larval stage. Like other eukaryotes, *C. elegans* obtains fatty acids from its diet and also synthesizes them de novo from acetyl-CoA (Perez and Van Gilst 2008). For de novo synthesis, all of the enzyme activities necessary for the synthesis of palmitic acid (16:0) from acetyl-CoA are encoded by two multifunctional enzymes: acetyl-CoA carboxylase and fatty acid synthase (Wakil 1989; Rappleye et al. 2003; Chirala and Wakil 2004; Wakil and Abu-Elheiga 2008). Palmitic acid can be integrated into TAGs or phospholipids, or it can be modified by fatty acid elongases and desaturases to form a variety of long chain polyunsaturated fatty acids (PUFAs; Wallis et al. 2002; Watts and Browse 2002). Long chain PUFAs are preferentially incorporated into membrane phospholipids, where they affect membrane properties including fluidity, temperature sensitivity, and signaling. In addition, *C. elegans* produces monomethyl branched-chain fatty acids (mmBCFAs), which are essential for growth and embryonic development (Kniazeva et al. 2004, 2012).

C. elegans Encodes Three SCD Orthologs

Due to the abundant and vital roles of lipids, the synthesis and breakdown of fatty acids are subject to many levels of regulation. A critical control point regulating lipid synthesis is the production of monounsaturated fatty acids. *C. elegans* encodes three stearoyl-CoA desaturases (SCDs), also known as Δ9 desaturases, that synthesize monounsaturated fatty acids from saturated fatty acids (Watts and Browse 2000). These enzymes, named FAT-5, FAT-6, and FAT-7, share significant amino acid similarity to rat and mouse SCDs, as well as to the yeast Δ9 desaturase OLE1. By expressing the SCD genes in the yeast *ole1* mutant, the substrate specificities of the three enzymes were determined. The FAT-5 desaturase is specific for palmitic acid (16:0), whereas the FAT-6 and FAT-7 desaturases preferentially introduce a double bond into stearic acid (18:0) (Watts and Browse 2000).

In contrast to mammals, oleic acid produced by the FAT-6 and FAT-7 SCDs is used as a substrate to produce a range of PUFAs. This reaction is mediated by a Δ12 desaturase, an enzyme normally found only in algae and plants (Wallis et al. 2002). Δ12 desaturation of oleic acid produces linoleic acid (18:2), which is further acted on by an omega-3 desaturase FAT-1 and a Δ6 desaturase FAT-3, which, together with elongation and Δ5 desaturation, produces a range of C18 and C20 omega-6 and omega-3 PUFAs (Fig. 14.1) (Watts and Browse 2002).

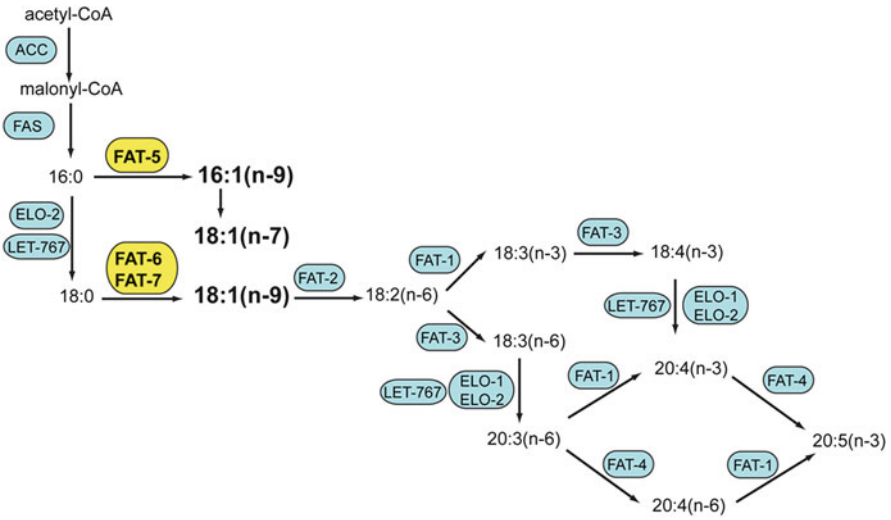

Fig. 14.1 Pathway of synthesis of unsaturated fatty acids in *C. elegans*. A variety of long chain polyunsaturated fatty acids can be synthesized from acetyl-CoA. The SCDs FAT-5, FAT-6, and FAT-7 are highlighted in *yellow*. *ACC* acetyl-CoA carboxylase, *FAS* fatty acid synthase, *ELO* fatty acid elongase, *LET* lethal, *FAT* fatty acid desaturase

Loss of One SCD Isoform Is Compensated for by Up-regulation of the Remaining SCDs

To determine the consequences of depleted SCD activity in *C. elegans*, loss of function mutants in each gene were isolated and analyzed. Gene knockdowns of individual SCD genes using RNAi have also been reported, but due to high sequence similarity between the three SCD genes, which lead to off-target effects, the phenotypes of strains carrying single and double mutations are more easily interpreted. The *fat-5;fat-6;fat-7* triple mutant strain was constructed and was found to be inviable, revealing that endogenous production of monounsaturated fatty acids is essential for survival (Brock et al. 2006). In contrast, the fatty acid composition changes and physiological consequences of the single desaturase mutants are subtle (Brock et al. 2006). The most highly expressed of the three Δ9 desaturases is the FAT-6 SCD, which is expressed in the intestine and hypodermal (skin) tissues (Brock et al. 2006). The *fat-6* mutants display increased stearic acid (18:0) and decreased unsaturated fatty acids, however, the change in fatty acid composition in the *fat-6* mutant worms is modulated by genetic compensation by the other SCDs. Real-time quantitative RT-PCR experiments showed that in the *fat-6* mutants, the *fat-7* and *fat-5* genes are up-regulated three to fivefold. Presumably, this compensation normalizes

the fatty acid composition and allows the strain to grow, reproduce, and behave like wild type (Brock et al. 2006).

Similarly, the *fat-7* mutants have wild type fatty acid composition, fat stores, and reproductive success, even when combined with *klf-3* mutants, which lack a Kruppel-like transcription factor and regulate fat storage in *C. elegans* and in humans (Brock et al. 2006; Zhang et al. 2011). In *fat-7* mutants, both *fat-6* and *fat-5* are up-regulated 1.5–2-fold (Brock et al. 2006). Knockdown of *fat-6* or *fat-7* by RNAi was reported to show decreased resistance to oxidative stress and increased heat tolerance (Horikawa and Sakamoto 2009). However, given that high degree of DNA sequence similarity (86 % identity) between the *fat-6* and *fat-7* cDNA sequences, off-target reduction of the other SCD homologues confounds the interpretation of these results.

In addition to genetic compensation, defects in fatty acid desaturase genes can be compensated for by dietary input. For example, the *fat-5* mutant has only subtle fatty acid composition changes when grown on plate with dietary *E. coli*. This is because palmitoleic acid (16:1) and its elongation product cis-vaccenic acid (18:1*n*-7) are provided by the *E. coli* diet. When *fat-5* mutants are grown axenically in media lacking *E. coli*, the *fat-5* mutants have a more profound fatty acid composition defect, showing greatly increased palmitic acid (16:0) and severely reduced cis-vaccenic acid (18:1*n*-7) (Brock et al. 2006). A recent study examining the relationship between fat stores and dietary restriction in *C. elegans* showed that *fat-5* expression increased two to fourfold in nematodes exposed to dietary restriction during development (Palgunow et al. 2012). Dietary restriction during development resulted in reduced body size, but an increase in the size of lipid droplets and in overall TAG stores. In addition to increases in *fat-5* expression, other lipogenic genes such as those encoding DGAT and acetyl-CoA synthetase were also up-regulated, suggesting that *C. elegans* adapts metabolically to conserve fat stores during periods of suboptimal nutriention (Palgunow et al. 2012).

In spite of compensation to normalize overall fatty acid composition, LC–MS analysis revealed differences in various TAG and PC species among the *fat-5*, *fat-6*, and *fat-7* mutant strains, as well as evidence of increased absorption of dietary fatty acids in the *fat-6* mutant (Castro et al. 2012). Interestingly, metabolomic analysis revealed significant differences in non-lipid metabolites such as alanine and succinate, indicating an increase in catabolism of amino acids and an increase in the activity of the TCA cycle in the *C. elegans* SCD mutants (Castro et al. 2012). Metabolomic studies such as these promise to provide insights as to the complex compensatory mechanisms that occur in animals when lipid homeostasis is altered.

Double Mutant Strains Reveal Additional Requirements for SCDs

Examination of double mutant strains reveals additional roles for monounsaturated fatty acid synthesis in temperature adaptation and in the regulation of fat storage. The *fat-5;fat-7*, *fat-5;fat-6*, and *fat-6;fat-7* double mutant strains display significant

changes in their fatty acid composition. The two strains lacking *fat-5* have increased palmitic acid (16:0) and severely reduced palmitoleic acid (16:1*n*-7) and cis-vaccenic acid (18:1*n*-7). Under standard conditions, these strains grow normally, but they have decreased survival in cold temperature (10 °C), indicating that the higher saturated and lower unsaturated fatty acid composition is detrimental to nematodes at low temperatures (Brock et al. 2007). In addition, the *fat-5;fat-6* double mutant strain was unable to survive starvation stress. After 4 days of starvation of L1 larvae in buffer without nutrients, wild type larvae are viable and maintain a normal fatty acid composition. However, the *fat-5;fat-6* double mutants die and have extremely high saturated fatty acid content in their membranes (Brock et al. 2007). The lack of dietary fatty acids, together with the observation that *fat-7* expression is highly repressed in the absence of food (Van Gilst et al. 2005b), reveals the roles of FAT-5 and FAT-6 in maintaining proper fatty acid composition during starvation.

The *fat-6;fat-7* Double Mutants Have Reduced Fat Stores and Increased Fat Oxidation, Similar to Mouse SCD1 Mutants

The *fat-6;fat-7* double mutant is unable to carry out normal PUFA synthesis, because it cannot synthesize the oleic acid precursor which is the substrate for the remaining PUFAs. However, unusual 18-carbon PUFAs are formed by elongation and desaturation of the FAT-5 product palmitoleic acid (16:1), which apparently partially substitute for vital functions performed by normal PUFAs (Brock et al. 2007). The *fat-6;fat-7* double mutants display slow growth and greatly reduced fertility. Dietary oleic acid partially, but not completely, rescues the growth and fertility deficiencies (Brock et al. 2007).

A striking phenotype of the *fat-6;fat-7* double mutant is greatly reduced fat stores compared to wild type (Brock et al. 2007). Real-time PCR analysis of fatty acid oxidation genes reveals that genes encoding components of mitochondrial fatty acid oxidation are expressed at higher levels in the *fat-6;fat-7* double mutant compared to wild type (Brock et al. 2007). Together with the SCD1 mouse observations, these studies demonstrate that Δ9 desaturase is an important determinant of TAG accumulation and that metabolic changes resulting from decreased SCD signaling elicit protection from obesity. Pharmacological manipulation of SCD activity might therefore benefit treatments of obesity, diabetes, and other diseases of metabolic syndrome. However, a better understanding of the downstream pathways affected by SCD1 signaling will be necessary in order to understand and anticipate potential side effects from such drugs (Dobrzyn and Ntambi 2005).

Nuclear Hormone Receptors Regulate Transcription of SCDs

Nuclear hormone receptors (NHRs) are important regulators of development and metabolism. Upon binding specific ligands, the NHRs dimerize and enter the nucleus where they activate or repress distinct target genes. Such regulation allows

for precise, reversible responses to environmental, developmental, and nutritional signals. *C. elegans* contains a hugely expanded repertoire of NHRs, and sequence analysis reveals that 269 out of 284 *C. elegans* NHRs evolved from an ancient form of the hepatocyte nuclear factor 4 (HNF4) family (Robinson-Rechavi et al. 2005; Taubert et al. 2011). Several NHRs have been implicated in the regulation of fat metabolism in *C. elegans*, and the SCDs are key gene downstream effectors. NHR-80 and NHR-49 are required for expression of the *fat-5, fat-6,* and *fat-7* genes (Van Gilst et al. 2005a; Brock et al. 2006). Both mutant strains have a high stearic acid (18:0) content and reduced oleic acid (18:1*n*-9). The expression of *fat-6* and *fat-7* was also shown to be decreased in *nhr-13* mutants, although the overall fatty acid composition was not affected (Pathare et al. 2012). In contrast, a mutation in the aryl-hydrocarbon receptor, *ahr-1*, results in increased expression of the *fat-7* gene, resulting in small, but statistically significant changes in fatty acid composition (Aarnio et al. 2010).

Both NHR-49 and NHR-80 are required for the up-regulation of *fat-7* needed to compensate for *fat-6* deletion mutants, and the compensation is essential because the *nhr-49;fat-6* and *nhr-80;fat-6* strains are not viable (Brock et al. 2006). Despite the similarities in their effects on Δ9 desaturation, *nhr-49* deletion mutants display more severe growth and reproductive defects than *nhr-80* mutants, probably because NHR-49 also regulates other lipid metabolism genes, including genes involved in the biosynthesis of sphingolipids, genes involved in β-oxidation, and other genes required for the response to nutrient deprivation (Van Gilst et al. 2005b; Pathare et al. 2012).

While mutations in *nhr-49* and *nhr-80* have additive defects in combination with mutations in SCDs, depletion of the *nhr-64* gene results in improved physiology of SCD mutants. The low fat stores, slow growth, and embryonic lethality phenotypes in *fat-6;fat-7* double mutants were improved in combination with *nhr-64*(*RNAi*) (Liang et al. 2010). Likely gene targets of NHR-64 that facilitated these improvements include β-oxidation genes, such as *ech-5* and a thiolase. However, other gene changes in *nhr-64*(*RNAi*) strains were also noted, including reduced expression of several acylCoA synthetase genes and increased expression of *pod-2* (acetyl-CoA carboxylase). These studies illustrate the complexity of compensatory mechanisms regulated by NHRs that balance fat storage, growth, and reproductive efficiency (Liang et al. 2010).

A network of regulators of metabolic genes enriched in NHRs has been experimentally mapped in *C. elegans,* implicating numerous NHRs in control of metabolic processes (Arda et al. 2010). It has been proposed that the expansion of NHRs in *C. elegans* evolved to enable rapid and adaptive responses to environmental clues, including dietary nutrients. It is likely that changes in SCDs are important for the adaptation to diets encountered in nature, which may vary significantly in their lipid content. Thus, the regulation of SCDs by numerous NHRs signifies their important physiological roles.

Regulation of *C. elegans* SCDs by SREBP

The sterol regulatory element binding protein (SREBP) family of basic helix–loop–helix zipper transcription factors are critical regulators of cholesterol and fatty acid homeostasis in mammals (Osborne and Espenshade 2009; Jeon and Osborne 2012). Newly synthesized SREBPs reside in the endoplasmic reticulum (ER) membrane and are inactive. When specific cellular lipid levels are low, the proteins are transported to the Golgi, where they are acted on by site 1 and site 2 proteases released by the N-terminal fragment of SREBP, which then enter the nucleus and activate transcription of lipogenic genes. Mammals express a family of SREBP isoforms (SREBP-1a, -1c, and SREBP-2), in which different isoforms are responsible for the expression of a unique set of lipid metabolism genes. For example, SREBP-2 mainly activates genes responsible for cholesterol uptake and synthesis, while SREBP-1 isoforms regulate genes involved in the biosynthesis of fatty acids, phospholipids, and TAGs. *C. elegans* possesses a single SREBP gene, *sbp-1*. Knockdown of *sbp-1* by RNAi leads to low fat stores, high saturated fatty acid content, impaired growth, and reduced expression of lipogenic genes, including the *fat-5, fat-6,* and *fat-7* SCDs (Kniazeva et al. 2004; Yang et al. 2006). A deletion mutant in the *C. elegans* SREBP homolog, *sbp-1*(*ep79*), displays a similar slow growth and low fat stores as the RNAi treatment (Liang et al. 2010).

The translocation of SREBP from the ER to the nucleus is well understood for the cholesterol-regulated SREBP-2 isoform (Brown and Goldstein 1997). When cholesterol is present, it binds to a cholesterol-sensing protein called SCAP, which promotes association with INSIG (Yang et al. 2002). This complex maintains SREBP in the ER membrane. When cholesterol is absent, INSIG no longer is complexed with SREBP, facilitating trafficking to the Golgi where proteases cleave the transmembrane domains, allowing for translocation to the nucleus (Sun et al. 2007). *C. elegans* does not synthesize cholesterol, and it does not encode an INSIG homolog; however, recent studies have shed light on a conserved mechanism for the nuclear translocation of the SREBP-1 isoform.

The mechanism of nuclear localization of the SREBP-1 isoform depends on membrane phosphatidylcholine (PC) levels. RNAi knockdown of genes encoding s-adenyslyl methionine transferase (*sams-1*) and other genes contributing to the PC synthesis pathway led to increased nuclear localization of SREBP and induction of SREBP target genes (such as *fat-5* and *fat-7*) in *C. elegans* and in mammalian liver cell lines (Walker et al. 2011). When PC is low, the Golgi protease enzymes undergo retrograde transport to the ER, where the proteases cleave the SREBP protein, releasing the transmembrane domains. This liberates the DNA binding portion of SREBP, allowing it to translocate into the nucleus, where it promotes transcription of lipid synthesis genes, including SCDs (Walker et al. 2011). Thus, when PC synthesis is disrupted, SREBP-induced gene expression changes led to increased fat stores in nematodes and hepatic steatosis (fatty liver) in the mouse. An independent study

confirmed that *C. elegans sams-1* and *pmt-1* mutants have high fat stores compared to wild type nematodes, and they express high levels of *fat-7* (Li et al. 2011).

During periods of food deprivation, lipid metabolism is shifted away from synthesis toward breakdown, and oxidation of fatty acids provides energy for the maintenance of the organism. Consistent with this, nuclear SREBP is rapidly depleted during fasting in the mouse liver (Horton et al. 1998) and in *C. elegans* (Walker et al. 2010). This is regulated by the NAD+-dependent deacetylase SIRT1, which directly deacetylates SREBP, leading to ubiquitination, protein degradation, and reduction in target gene expression. The importance of SREBP regulation of the FAT-7 SCDs is apparent, as the expression of the *fat-7* SCD is especially sensitive to fasting, with undetectable transcript levels in the absence of food (Walker et al. 2010).

MDT-15 Interacts with SBP-1 and NHRs to Regulate *C. elegans* SCDs

After binding to target sequences in specific promoters, SREBP and NHRs recruit multi-protein coregulator complexes containing transcriptional cofactors that link the transcriptional activator to the transcription initiation machinery (Blazek et al. 2005). One component, the ARC/mediator subunit MDT-15/MED15, interacts directly and specifically with the activation domain of SREBP (Yang et al. 2006). The *C. elegans* MDT-15 also binds specifically to NHR-49 and NHR-64 (Taubert et al. 2006), which both regulate the expression of genes involved in lipid metabolism. Accordingly, RNAi reduction of *mdt-15* expression results in slow growth, low fat stores, and reduced expression of *fat-7*. Dietary supplementation with oleic acid rescues many of the defects of *sbp-1* and *mdt-15* RNAi animals, indicating that the SCDs are key targets of these regulators (Yang et al. 2006). MDT-15 is also important for broader functions, including induction of detoxification genes in response to certain ingested xenobiotics and heavy metals, suggesting that it interacts with additional transcription factors (Taubert et al. 2008).

FOXO Regulates SCDs to Confer Long Lifespan and Cold Tolerance

Insulin/IGF-1 signaling modulates development, metabolism, stress resistance, and longevity in *C. elegans* (Lin et al. 1997, 2001; Henderson and Johnson 2001). Insulin/IGF-1 signaling during favorable growth conditions negatively regulates the DAF-16/FOXO transcription factor by a phosphorylation cascade that prevents nuclear localization. During unfavorable conditions, such as low nutrients, UV irradiation, certain toxins, and exposure to high temperatures, the phosphorylation cascade is inhibited such that the DAF-16/FOXO transcription factor enters the nucleus,

leading to the expression of many protective genes, such as superoxide dismutase, drug metabolizing enzymes, and molecular chaperones (McElwee et al. 2003; Murphy et al. 2003). This altered gene expression confers long lifespan and stress resistance to the nematodes. SCDs have been identified as targets of DAF-16/FOXO (Murphy et al. 2003; Schuster et al. 2010), and *fat-7*(*RNAi*) leads to a shortened lifespan in the long-lived insulin/IGF-1 receptor mutant *daf-2* (Murphy et al. 2003). A recent study shows that the *age-1* mutant, a long-lived mutant disrupted in the insulin/IGF-1 signaling pathway, is cold-tolerant, and this tolerance depends on DAF-16/FOXO (Savory et al. 2011). While cold stress does not induce nuclear localization of DAF-16/FOXO, cold tolerance in the *age-1* mutants depends on functional SCDs. SCDs have previously been shown to be important for adaptation to cold temperatures in many organisms including plants (Browse and Xin 2001), fish (Tiku et al. 1996), and *C. elegans* (Brock et al. 2007; Murray et al. 2007). Increased SCD expression leads to higher unsaturated fatty acid content, resulting in increased membrane fluidity that is necessary for optimal membrane function in cold environments.

Fatty Acid Desaturation and Other Lipid Modifications in Germ Cells Regulate Lifespan

In *C. elegans*, ablation of germ cells results in a long lifespan. This can be achieved through laser ablation of germ cell precursors or by mutations that block the proliferation of germ cells (Hsin and Kenyon 1999; Arantes-Oliveira et al. 2002). Recent studies reveal that fat metabolism is altered in worms lacking germ cells and that the changes in fat metabolism are required for the increased longevity in these strains (Wang et al. 2008; O'Rourke et al. 2009; Goudeau et al. 2011). Specifically, the lipase *lipl-4* is induced in worms lacking a germline, suggesting that lipase activity might underlie the lifespan extension observed in germline-less animals. In agreement with this, depletion of *lipl-4* shortens the lifespan of worms lacking germ cells (Wang et al. 2008), while overexpression of *lipl-4* is sufficient to extend lifespan in wild type worms (Wang et al. 2008; Lapierre et al. 2011). Autophagy, the process of sequestering and degrading cytosolic components is also increased in germline-less *C. elegans*. The FOXA transcription factor PHA-4 is required for the induction of autophagy-related genes (Lapierre et al. 2011). The process of autophagy mediated through PHA-4 is required for extended lifespan of the germline-less worms, as well as for sustained induction of the *lipl-4* gene (Lapierre et al. 2011).

A recent study demonstrated that SCD activity under the control of NHR-80 is required for the extended lifespan of germline-less worms (Goudeau et al. 2011). The study found that a mutation in *nhr-80* blocks the extended lifespan of a germline-less *glp-1* mutant, while overexpression of *nhr-80* further increases the extended lifespan of *glp-1*. Neither the *nhr-80* mutation nor overexpression of *nhr-80* affected the lifespan of wild type worms (Goudeau et al. 2011). The SCD activity is

required for the extension of lifespan because there was no lifespan extension in the *glp-1;fat-6;fat-7* triple mutants, even in strains where the *nhr-80* gene was ectopically overexpressed. This discovery delineates a link between signals from the reproductive system, SCD activity, and longevity. Further studies will be necessary to determine the key physiological roles of SCDs in longevity, where they may be necessary for optimal membrane fluidity, efficient energy storage, or as precursors of lipid signaling molecules.

Conclusions

SCD activity is essential for proper lipid homeostasis in *C. elegans*. SCDs play important physiological roles in ensuring proper membrane fluidity, synthesizing precursors of PUFAs and other lipid signaling molecules, and enabling efficient fat storage. Whole organism approaches have facilitated studies of the consequences and compensatory mechanisms that occur as a result of reduced SCD activity, including the importance of conserved SCD regulators such as nuclear receptors and SREBP. Future studies promise to clarify lipid-mediated mechanisms contributing to the complex processes of development, reproduction, stress responses, and aging.

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