Lipid and Carbohydrate Metabolism in *Caenorhabditis elegans*

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ABSTRACT Lipid and carbohydrate metabolism are highly conserved processes that affect nearly all aspects of organismal biology. *Caenorhabditis elegans* eat bacteria, which consist of lipids, carbohydrates, and proteins that are broken down during digestion into fatty acids, simple sugars, and amino acid precursors. With these nutrients, *C. elegans* synthesizes a wide range of metabolites that are required for development and behavior. In this review, we outline lipid and carbohydrate structures as well as biosynthesis and breakdown pathways that have been characterized in *C. elegans*. We bring attention to functional studies using mutant strains that reveal physiological roles for specific lipids and carbohydrates during development, aging, and adaptation to changing environmental conditions.

KEYWORDS *Caenorhabditis elegans*; ascarosides; glucose; fatty acids; phospholipids; sphingolipids; triacylglycerols; cholesterol; maradolipids; WormBook

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INTEREST in lipid and carbohydrate metabolism has skyrocketed in recent years along with the human obesity and diabetes epidemics. All animals must eat to survive and reproduce, and dietary macronutrients are vital precursors required to build cellular material for growth and reproduction, even though overnutrition leads to a variety of modern diseases in humans. In recent years, there has been a growing appreciation of the dynamic roles of fat depots and the regulation of energy homeostasis, especially the interplay between the nervous system and various metabolic pathways. *Caenorhabditis elegans* is best known for powerful genetic and genomic analysis dissecting mechanisms of development, nervous system function, and aging. These studies have contributed to an appreciation of the roles of macronutrients and conserved metabolic pathways in these vital biological processes. The sections on *Fatty Acids*, *Storage Lipids*, *Membrane Lipids*, and *Sterol and Prenol Lipids* of this review outline lipid structures and metabolic pathways leading to the synthesis and metabolism of a wide range of lipids and describe specific functions for lipids in development and aging. *Introduction to Carbohydrates* and *Functions and Metabolism of Monosaccharides* introduce carbohydrate structures, metabolic pathways, and functions for simple and complex carbohydrates in development, aging, and protection from environmental stressors.

Lipids are small organic molecules that are insoluble in water, but are soluble in organic solvents. Biochemically, they originate entirely or in part from carbanion-based condensations of thioesters, forming fatty acids, which are components of triacylglycerols (TAGs), phospholipids, and sphingolipids; or by carbocation-based condensation of isoprene units, forming isoprenol derivatives including sterols (Fahy *et al.* 2009)

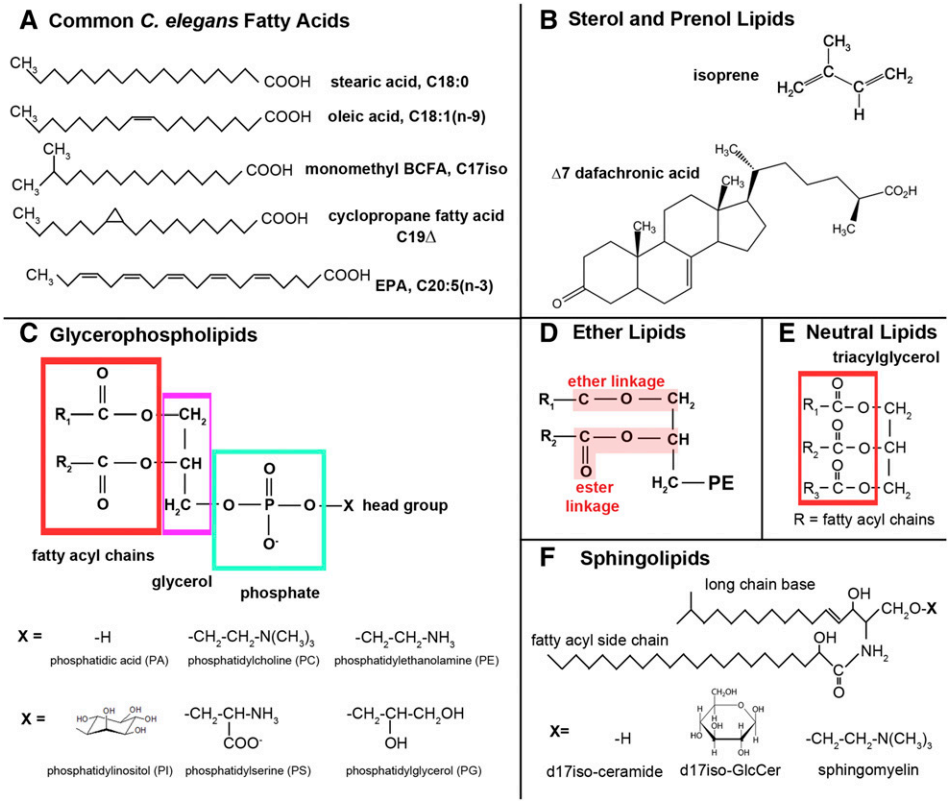

Figure 1 Structures of *C. elegans* lipid classes. (A) Fatty acids. (B) Sterol and prenol lipids. (C) Glycerophospholipids. Fatty acyl groups are outlined in red, the glycerol backbone in pink, and the phosphate group is outlined in blue, head group structures abbreviated as X are shown below. (D) A PE structure shows an ether linkage at C1 and an ester linkage at C2. (E) TAG molecule showing the fatty acyl chains outlined in red. (F) Sphingolipid structure showing the branched long chain base, head group structures abbreviated as X are shown below.

(Figure 1). *C. elegans* has the potential to synthesize thousands of distinct lipid species, many of which have yet to be characterized.

Lipids perform many essential functions in the cell. Their highly reduced state renders them efficient energy storage molecules. They are the hydrophobic units of bilayers that form cellular and organellar membranes, and they are potent signaling molecules. In membranes, the diversity of lipid species contributes to cellular and organellar functions. For example, in most organisms, the outside leaflet of the plasma membrane is composed of entirely different lipid species than the inside leaflet (Shevchenko and Simons 2010). Plasma membrane lipid composition differs among various tissues, as does lipid composition of endoplasmic reticulum (ER), mitochondria, and other cellular organelles (van Meer *et al.* 2008). Because the distinct lipid composition is actively maintained by ATP-consuming enzymes, it is believed that the lipid asymmetry is important for distinct cellular functions. Additionally, specific lipid composition influences membrane protein folding and topology (Vitrac *et al.* 2015). Questions regarding functions of distinct lipids in membranes or lipid requirements for protein functions are only beginning to be addressed in *C. elegans*. To date, research in the *C. elegans* lipid field has been ongoing to characterize lipid biosynthetic genes, regulatory genes affecting lipid synthesis, storage, and breakdown, as well as the neurological and developmental consequences that occur when specific lipids are not synthesized or correctly or when lipid homeostasis is incorrectly regulated.

Fatty Acids, *Storage Lipids*, *Membrane Lipids*, and *Sterol and Prenol Lipids* will follow the LIPID MAPS classification scheme (Fahy *et al.* 2009) to review the current state of knowledge concerning the biosynthesis, breakdown, and functions of specific lipid classes in *C. elegans*. Although 471 putative lipid metabolism genes have been identified based on the KEGG database and homology to mammalian lipid metabolism (Zhang *et al.* 2013), this review will focus on genes that have been experimentally verified based on biochemical assays, changes in nematode lipid composition in loss of function strains, or functional phenotypes in *C. elegans*. Roles for specific lipids have often been identified in forward or reverse genetic screens, which offer unbiased determinations of functions of specific lipid classes (Han 2015; Zhang *et al.* 2015). Methods for analysis of specific lipids will be mentioned briefly in the appropriate sections, and more information regarding lipidomics analysis methods can be found in a recent comprehensive review (Witting and Schmitt-Kopplin 2016).

Fatty Acids

Characteristics of C. elegans fatty acids

Fatty acids are carboxylic acids with long aliphatic chains, which in *C. elegans* lipids contain 14–20 carbons (Tanaka *et al.* 1996; Watts and Browse 2002) (Figure 1A). Fatty acids are the building blocks and precursors for storage lipids (TAGs), membrane lipids (phospholipids and sphingolipids),

and signaling lipids (fatty acyl amides, eicosanoids, and others). Fatty acids can be saturated, containing no double bonds, monounsaturated, containing one C–C double bond, or polyunsaturated, containing two or more C–C double bonds. *C. elegans* double bonds are in the "cis" configuration, and are methylene interrupted, meaning double bonds are spaced along the carbon chain in intervals of three carbons. Fatty acids are named by common (trivial) names, systematic chemical names, and chemical abbreviations. For example, the systematic name of linoleic acid is 9,12 octadecanoic acid, which is abbreviated 18:2 (18 carbons with two double bonds). Further information regarding double bond location is given by the designation n-6 or ω6, which indicates the position of the terminal double bond. Thus, using the abbreviations 18:3n-3 and 18:3n-6, α-linolenic (9,12,15-octadecatrienoic) can be distinguished from γ-linolenic (6,9,12-octadecatrienoic). *C. elegans* differs from most animals in that it has the capacity to synthesize a range of monomethyl and polyunsaturated fatty acids starting with acetyl-CoA or isobutyryl-CoA as precursors. In contrast, most animals must obtain polyunsaturated fatty acids from their diet, because they do not possess the enzymes to convert monounsaturated fatty acids to polyunsaturated fatty acids (Wallis *et al.* 2002).

Methods to analyze fatty acid composition

Fatty acids from worm populations are commonly analyzed using acidic methylation to produce fatty acid methyl esters, which are separated from each other by gas chromatography (GC), and detected with either mass spectrometry (MS) or flame ionization detection (FID) (Watts and Browse 2002). This is a sensitive method, because use of a polar column such as SP-2340 or Omegawax aids in the separation of fatty acyl isomers with identical chemical composition but different double bond positions. Because *C. elegans* synthesizes and accumulates several isomers of 18:1 and 20:4, it is important to use authentic standards to distinguish among isomers during analysis.

When using single quad GC/MS analysis to characterize the fatty acid composition of distinct lipid classes, such as triglycerides or phospholipids, the lipids must first be separated before GC/MS analysis of fatty acids. One method to separate neutral, glycerophospholipids, and sphingolipids is using a solid state column (Perez and Van Gilst 2008). Another method, thin layer chromatography (TLC), is useful because distinct phospholipid species as well as neutral lipids can be separated using a two-solvent system (Shi *et al.* 2013). After solid state or TLC separation, the fatty acyls can then be derivatized using acidic methylation for GC/MS analysis. The disadvantage of the solid state or TLC method for lipid separation is that it requires large amounts of material, typically 10,000 young adult *C. elegans* per separation.

Fatty acid composition of intact lipids can be analyzed by liquid chromatography/mass spectrometry (LC/MS) (Entchev *et al.* 2008; Castro *et al.* 2012). The use of triple quad mass spectrometers and shotgun lipidomics allows lipid analysis without separating lipid classes (Witting and Schmitt-Kopplin 2016). In *C. elegans*, due to the large number of fatty acyl classes, this has the potential to generate over 1000 distinct lipid species (Entchev *et al.* 2008; Shi *et al.* 2016; Witting and Schmitt-Kopplin 2016). Methods to analyze intact lipids are required to identify sphingolipids and ether-linked lipids, because the acidic methylation technique does not liberate the fatty acyl of the sphingosine base or the alkyl group on the ether-linked lipid. A disadvantage of intact lipid analysis methods for *C. elegans* fatty acids is the inability to identify distinct isomers. For example, the cyclopropane fatty acids derived from *Escherichia coli* are simply identified by their mass as 17:1 or 19:1, which has propagated the myth that *C. elegans* synthesizes a considerable amount of odd chain fatty acids, when in fact these represent mmBCFA C17iso or dietary cyclopropane fatty acids (C17Δ and C19Δ). Only very small amounts of straight, odd-chain fatty acids accumulate in *C. elegans* lipids. Another disadvantage of intact lipid analysis methods is that all species are identified based on their total mass, making the identification of specific fatty acyl groups ambiguous; for example, PC-40:8 could contain one 20:3 and one 20:5, or two 20:4 (which could be either the 20:4n-3or the 20:4n-6 isomers).

C. elegans fatty acids are synthesized de novo, or are derived from dietary fatty acids

Stable isotope labeling studies using dietary ^{13}C have been used to study synthesis and turnover of fatty acids (Perez and Van Gilst 2008; Dancy *et al.* 2015); ~7–20% of *C. elegans* fatty acids are synthesized *de novo* from acetyl-CoA, with the exception of the monomethyl fatty acids, which cannot be derived from *E. coli* precursors and are 100% synthesized when *C. elegans* feeds on *E. coli* OP50 (Perez and Van Gilst 2008). The remaining fatty acids are incorporated or modified from *E. coli* fatty acids. Fatty acyl turnover is rapid, with ~4.5% of membrane fatty acid acyl components of phospholipids being turned over every hour, and 2.7% of neutral (storage) lipids being turned over every hour (Dancy *et al.* 2015). This means that the majority of fatty acyl components of membrane lipids are replaced daily. When grown on nematode growth media (NGM) plates to stationary phase, *E. coli* OP50 and HT115 membranes are composed of ~37% saturated fatty acid (31% 16:0, 6% 14:0, and trace amounts of 18:0), 11% monounsaturated fatty acids, (trace amounts of 16:1n-7 and 11% 18:1n-7), and 49% cyclopropane fatty acids (Brooks *et al.* 2009). The *E. coli* HB101 strain contains considerably more monounsaturated fatty acids, including 16:1n-7 and 18:1n-7, and reduced amounts of cyclopropane fatty acids (Brooks *et al.* 2009). In worms, the relative composition of cyclopropane fatty acids and 18:1n-7reflects the dietary *E. coli* source (Brooks *et al.* 2009). Neither strain of *E. coli* produces monomethyl branch chain fatty acids or polyunsaturated fatty acids.

The *de novo* synthesis of fatty acyl chains using the two-carbon subunit acetyl-CoA is achieved by the activity of fatty acid synthase (FAS). This is a multi-enzyme protein encoded by one large gene (*fasn-1*) and contains six catalytic

activities, including beta-ketoacyl synthase, acetyl/malonyl transacylase, beta-hydroxyacyl dehydratase, enoyl reductase, β-ketoacyl reductase, acyl carrier protein, and thioesterase (Chirala and Wakil 2004). This reaction requires NADPH, and the condensation of malonyl-CoA and the following reaction series are repeated seven times to generate palmitate (16:0) (Figure 2).

The rate-limiting step of *de novo* fatty acid synthesis is the generation of malonyl-CoA by acetyl-CoA carboxylase (ACC), encoded by the *pod-2* gene in *C. elegans* (Rappleye *et al.* 2003). The ACC enzyme requires biotin, and uses energy generated by the hydrolysis of ATP to catalyze the carboxylation of acetyl-CoA to malonyl-CoA (Waite and Wakil 1962). Malonyl CoA is the substrate for each round of fatty acid synthesis, with CO_2 being liberated during each condensation reaction. After *de novo* synthesis of 16:0, malonyl-CoA is used as the substrate for the elongation of fatty acids, which uses separate condensation, reductase, dehydratase, and thiosterase enzymes, including the ELO-1,ELO-2, and ELO-3 synthases (Watts and Browse 2002; Kniazeva *et al.* 2004; H. Zhang *et al.* 2011), the LET-767 β-ketoacyl dehydratase (Entchev *et al.* 2008), and likely the HPO-8 β-hydroxyacyl dehydratase, and the ART-1 enoyl reductase.

Monomethyl branch chain fatty acids serve as precursors of sphingolipids

An understudied family of fatty acids containing a methyl branch is synthesized by bacteria and is a significant component of the human diet, especially of consumers of dairy and ruminant meat (Ran-Ressler *et al.* 2014). *C. elegans* synthesize monomethyl branch chain fatty acids and these play important functions in the organism (reviewed in Han 2015). The pathway to synthesize monomethyl branched chain fatty acids (mmBCFAs) requires ACC and FAS, similar to the synthesis of straight chain fatty acids, except that the starting material is isovaleryl-CoA, which is derived from the branched chain amino acid leucine (Kniazeva *et al.* 2004) (Figure 2). The biosynthesis pathway involves the branched-chain ketoacid dehydrogenase complex (BCKDC), fatty acid synthase (FASN-1), acetyl-coA carboxylase (POD-2), fatty acyl elongases (ELO-5 and ELO-6), the LET-767 β-ketoacyl dehydratase, and an acyl CoA synthetase (ACS-1), After five rounds of condensation of malonyl-CoA by FAS, the ^{13}C-iso fatty acids are elongated by activities of *elo-5*, *elo-6*, and *let-767* to form 13-methyl myristic acid (C15iso) and 15-methyl hexadecanoic acid (C17iso), which accumulate in *C. elegans* storage lipids, glycerophospholipids, and sphingolipids (Kniazeva *et al.* 2004; Entchev *et al.* 2008; Zhu *et al.* 2013; Jia *et al.* 2016).

The *elo-5* mutants, which lack mmBCFA, arrest as L1 larvae with a phenotype similar to starvation arrest (Kniazeva *et al.* 2004, 2008). Involvement with the insulin signaling pathway and the correlation with 17iso in TAG and fat stores indicate that accumulation or synthesis of these fatty acids could indicate nutrient status (Kniazeva *et al.* 2008; Brooks *et al.* 2009). The larval arrest phenotype is suppressed for one generation by mutations in *tat-2*, which encodes a P-type

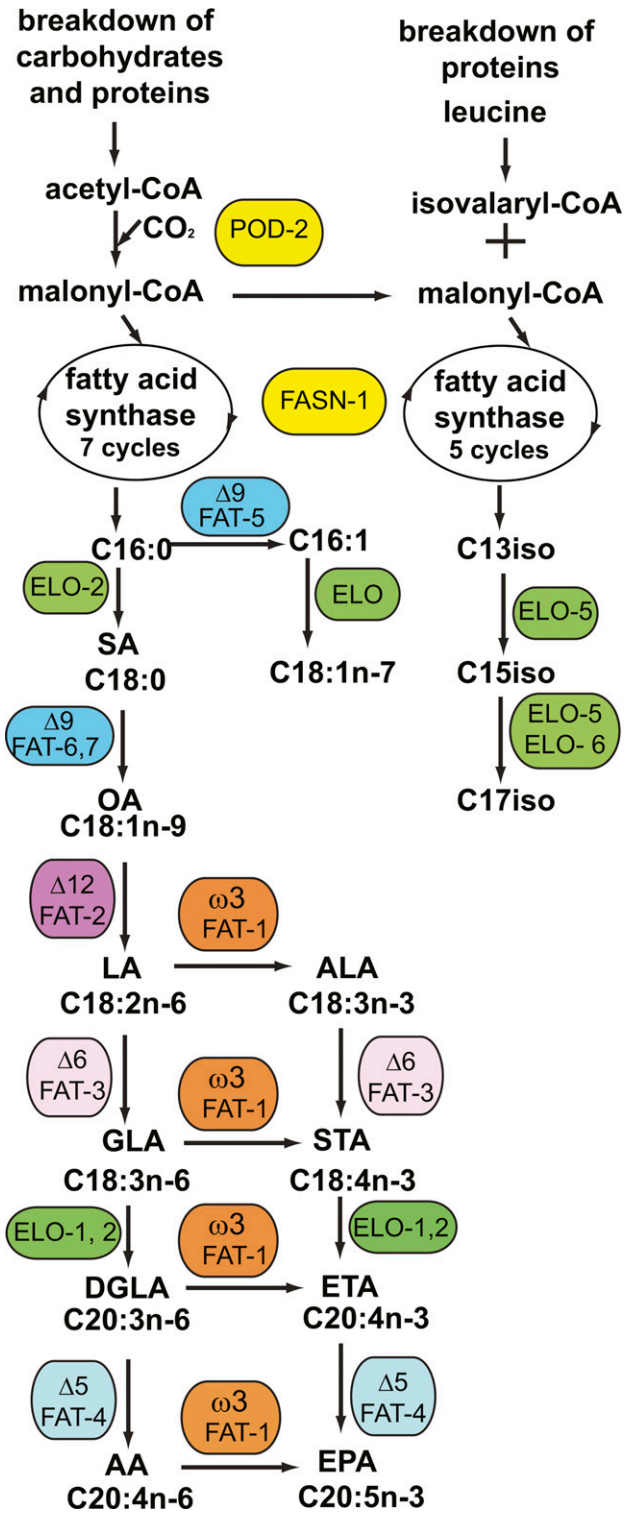

Figure 2 Pathway of *de novo* fatty acid synthesis in *C. elegans*. Enzyme names and activities are enclosed in ovals. ELO, elongase; C13iso, 11-methyldodecanoic acid; C15iso, 13-methyltetradecanoic acid; C17iso, 15-methylhexanoic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; ALA, alpha linoleic acid; GLA, gamma linoleic acid; STA, stearidonic acid; DGLA, dihommo gamma linoleic acid; ETA, eicosatetraenoic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid.

ATPase/flippase that likely allows for the translocation of lipids within the bilayer or between organelles (Seamen *et al.* 2009). In addition, a mutation in a peroxisomal regulatory gene partially restored development as well as mmBCFA levels (Wang *et al.* 2013).

While C15iso and C17iso are found in several lipid species, including TAGs, PC, and PE, an important role for mmBCFA are as structural components of sphingolipids. All *C. elegans* sphingolipids contain C17iso as the long chain acyl component of the sphingoid base (Chitwood *et al.* 1995; Zhu *et al.* 2013). During sphingolipid biosynthesis, the mmBCFA C15iso condenses with the amino acid serine to form an unusual 17-carbon branched chain sphingoid base. This is different from mammals, which typically synthesize the sphingoid base from palmitic acid (16:0) and serine. The specialized sphingoid base is important for function, because the developmental phenotypes of *elo-5* mutants depend on C17iso base of sphingolipids (Zhu *et al.* 2013). More information on sphingolipid synthesis and function is found below in *Sphingolipid biosynthesis and function*.

Dietary and endogenous synthesized monounsaturated fatty acids regulate fat accumulation and longevity

Monounsaturated fatty acids are abundant in *C. elegans* lipids, especially *cis*-vaccenic acid (18:1n-7), which can be directly obtained from the *E. coli* diet. Alternatively, monounsaturated fatty acids can be produced from saturated fatty acid precursors by desaturation. Desaturase enzymes are diiron proteins, which use conserved histidine residues to align the iron and cytochrome b_5 to extract protons from acyl chains (Shanklin *et al.* 1994). Desaturase enzymes are characterized by specificity for the position along the acyl chain. Stearoyl-CoA desaturase, or Δ 9 desaturase, insert the first double bond at carbon 9 into a saturated fatty acyl chain, and are among the most extensively studied desaturases in mammals. *C. elegans* encodes three Δ9 desaturases. The FAT-5 desaturase is specific for 16:0, producing 16:1n-7, which can then be elongated to 18:1n-7 (*cis*-vaccenic acid) (Watts and Browse 2000). The FAT-6 and FAT-7 desaturases mainly act on 18:0, producing oleic acid, 18:1n-9 (Watts and Browse 2000). Unlike in mammals, in *C. elegans* 18:1n-9 can be further desaturated and elongated to form polyunsaturated fatty acids, thus 18:1-9 only accumulates to a small degree in *C. elegans* membranes and neutral lipids, even though it is a much more significant component of mammalian membranes (Wallis *et al.* 2002). In spite of dietary MUFA provided by the *E. coli* diet, endogenous production of monounsaturated fatty acids is essential, as the *fat-5*;*fat-6*;*fat-7* triple mutant strain is lethal (Brock *et al.* 2006).

In mammals and in *C. elegans*, reduction of Δ9 desaturase activity leads to reduced fat accumulation, and thus Δ9 desaturases are considered to be lipogenic enzymes (Ntambi *et al.* 2002; Brock *et al.* 2007; Sampath and Ntambi 2011). In mammals, monounsaturated fatty acids, such as oleic acid, are the preferred substrate for acyl transferase enzymes that synthesize triglycerides (Cases *et al.* 2001). However, in *C. elegans*, triglyceride fatty acids consist mainly of dietary fatty acids obtained from *E. coli* (Perez and Van Gilst 2008; Brooks *et al.* 2009). In both mammals and *C. elegans*, stearoyl-CoA desaturase knockdown leads to increased fatty acid oxidation (Flowers and Ntambi 2008; Shi *et al.* 2013), which can mitigate lipotoxicity caused by excess saturated fatty acids (Listenberger *et al.* 2003).

Because the Δ12 desaturase activity allows polyunsaturated fatty acids (PUFAs) to be synthesized from monounsaturated fatty acids, the Δ9 desaturases are vital for providing precursors for further desaturation and elongation. In addition to this role, the *fat-6*;*fat-7* double mutants show reduced turnover of fatty acyl groups, which may be necessary for optimal healthspan and longevity (Dancy *et al.* 2015). In a more specific context, the Δ9 desaturases have been implicated in a signaling event that regulates long lifespan in nematodes lacking a germ line. The long lifespan depends on two nuclear hormone receptors, NHR-80 and NHR-49, both of which regulate Δ9 desaturase activity (Van Gilst *et al.* 2005a; Brock *et al.* 2006; Goudeau *et al.* 2011; Ratnappan *et al.* 2014). In addition to Δ9 desaturation, the longevity of germline-less nematodes depends on a functional autophagy pathway, which is described in *Neutral lipid catabolism*. Finally, chromatin modification in the germ line also influences Δ9 desaturation, and mutants defective in H3K4me3 transferase activity have a long lifespan and increased monounsaturated fatty acid levels (Han *et al.* 2017).

Saturated fatty acid levels and temperature adaptation

C. elegans lipids contain relatively low levels of saturated fatty acids compared to mammalian cells, with ~5% of fatty acids in total lipid extracts consisting of 16:0, 6% 18:0, and small amounts (<2%) of 14:0 and 20:0 (Tanaka *et al.* 1996; Watts and Browse 2002). The addition of just one double bond to a saturated fatty acyl chain changes the melting temperature of the fatty acid drastically. The homeoviscous theory of membranes adaptation states that membrane saturation levels are regulated in order to adapt to environmental temperature changes (Sinensky 1974). It appears that low levels of saturated fat found in *C. elegans* lipids likely allow for growth in cool environments, because the Δ9 desaturase gene *fat-7* is induced upon transfer to cold temperature (Murray *et al.* 2007), and *C. elegans fat-6*;*fat-7* double mutants, which accumulate high levels of saturated fatty acids, are cold sensitive (Brock *et al.* 2007), as are *fat-5*;*fat-7*(*RNAi*) (Murray *et al.* 2007). Other cold-sensitive and cold-tolerant mutant strains also influence the activity of Δ9 desaturation, including the phosphoinositide-3 kinase mutant *age-1* (Savory *et al.* 2011), the adiponectin receptor homolog *paqr-2* (Svensson *et al.* 2011; Svensk *et al.* 2013), and *iglr-2* (Svensk *et al.* 2016). Mutations in the acyl-CoA dehydratase gene *acdh-11* were found to suppress *paqr-2* by upregulating *fat-7* (Ma *et al.* 2015). Structural studies of ACDH-11 suggest that short chain fatty acids become sequestered by binding to ACDH-11, and that the sequestration of these fatty acids inhibits expression of *fat-7*, reducing fatty acid desaturation such that

Table 1 Fatty acid composition of isolated lipid classes in wild type *C. elegans*

	15iso	16:0 (%)	17iso	18:0DMA	17Δ (%)	18:0 (%)	18:1n-9 (%)	18:1n-7 (%)	18:2 (%)	19Δ	20:3 (%)	20:4	20:4n-3	20:5
PC	2.0%	2.3	1.5%	0.1%	8.4	2.2	2.3	8.4	6.4	15.0%	4.7	3.0%	7.2%	32.8%
PE	1.8%	7.7	6.3%	7.5%	9.2	14.3	2.1	12.9	6.3	12.4%	3.4	1.3%	3.5%	9.3%
PI	0.7%	4.3	1.3%	0.1%	1.6	26.8	1.9	8.6	1.9	13.5%	1.2	1.8%	1.6%	35.1%
PS	0.5%	5.6	3.8%	0.4%	3.1	26.4	3.0	7.7	3.3	5.0%	6.6	3.2%	5.1%	26.1%
CL	ND	6.5	ND	ND	2.4	8.9	1.3	4.9	2.8	ND	21.5	ND	44.9%	4.4%
TAG	5.2%	4.5	3.9%	0.2%	32.2	2.9	6.0	8.5	2.9	23.5%	1.7	0.5%	0.9%	2.6%
Total lipids	3.3%	4.1	3.5%	2.0%	20.3	5.2	3.9	9.9	4.6	17.9%	3.4	1.6%	4.0%	12.5%

Sources for fatty acid composition: Synchronized, young adult stage N2 hermaphrodites grown at 20° were used for these measurements. For all lipids except CL, values are average from Shi *et al.* (2013), (2016); for CL, values are from Hou *et al.* (2014). ND, Not detected; 15iso, 13-methyltetradecanoic acid; 17:iso, 15-methylhexanoic acid; 18:0DMA, 18:0 dimethylacetal, formed from 18:0 with vinyl ether linkage; 17Δ, *cis*-9,10-methylenehexadecanoic acid; 19Δ, *cis*-11,12-methylene octadecanoic acid, PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; CL, cardiolipin; TAG, triacylglycerols.

membranes become more saturated in response to increased temperature (Ma *et al.* 2015).

Cyclopropane fatty acids are obtained from the diet and are not essential

C. elegans grown on NGM plates with *E. coli* (OP50) contain large amounts of cyclopropane fatty acids in their lipids. These fatty acids are formed in bacteria by addition of a methylene group across the double bond of an unsaturated fatty acid by the enzyme CFA synthase, and they accumulate during stationary phase of *E. coli* growth (Grogan and Cronan 1997). *E. coli* strains with fewer cyclopropane fatty acids accumulate more unsaturated fatty acids, and vice versa (Brooks *et al.* 2009). The two cycloproane fatty acids found in *E. coli* and *C. elegans* are *cis*-9,10-methylenehexadecanoic acid, abbreviated C17Δ, and *cis*-11,12-methyleneoctadecanoic acid, abbreviated C19Δ (Figure 1A). In *C. elegans* lipids, the majority of cyclopropane fatty acids are components of triglyceride storage lipids, and are incorporated in membrane phospholipids to a lesser degree (Table 1). *C. elegans* grown on CFA synthase mutants of *E. coli* are viable and do not contain cyclopropane fatty acids in lipid extracts (Kaul *et al.* 2014). This demonstrated that *C. elegans* do not synthesize cyclopropane FAs, and that cyclopropane FA are not essential for *C. elegans*. The same study also showed that *C. elegans* recover more efficiently from dauer arrest in the absence of cyclopropane fatty acids than in their presence (Kaul *et al.* 2014), suggesting that, for some processes, dietary cyclopropane fatty acids may be detrimental.

PUFAs are required for growth, reproduction, and neurotransmission

A unique aspect of *C. elegans* fatty acid metabolism is the ability to synthesize a wide range of PUFAs *de novo*. There are four fatty acid desaturases that convert 18:1n-9 into a range of C18 and C20 PUFAs. The FAT-2 (Δ12) desaturase catalyzes the conversion of oleic acid (18:1n-9) into the PUFA linoleic acid (18:2n-6), and the FAT-1 desaturase catalyzes the conversion of 18-carbon and 20-carbon omega-6 fatty acids into omega-3 fatty acids (Figure 2) (Spychalla *et al.* 1997; Peyou-Ndi *et al.* 2000; Watts and Browse 2002). The FAT-1 and FAT-2 desaturases are similar to the plant desaturases Δ12 and omega-3 desaturases encoded by FAD2, FAD3, FAD6, FAD7, and FAD8, and homologs are not normally present in animals, although they have been identified in other nematode species. Two *C. elegans* desaturases, the Δ5 desaturase FAT-4 and the Δ6 desaturase FAT-3 are homologs of human FADS1 and FADS2, respectively (Napier *et al.* 1998; Watts and Browse 1999; Marquardt *et al.* 2000). The fatty acid desaturases function as part of an electron transport pathway requiring cytochrome b_5 and a cytochrome b_5 reductase to extract two protons from an acyl chain. Fatty acid composition analysis indicates that *hpo-19* and T05H4.4 encode cytochrome b_5 reductases that act in this pathway (Y. R. Zhang *et al.* 2016).

The sum of the C18 and C20 PUFAs comprise ~28% of fatty acids in total worm lipids (Shi *et al.* 2013). PUFAs are not distributed evenly in worm lipids, for example, triacylglycerol storage lipids contain relatively low levels of PUFAs (9% of total fatty acids), while phosphatidylcholine (PC) consists of 56% PUFAs (Shi *et al.* 2013) (Table 1). The most abundant PUFA in all *C. elegans* lipid classes is eicosapentaenoic acid (EPA, 20:5)—the final fatty acid in the desaturation pathway (Figure 1A and Figure 2). Overall, PUFA levels, especially 20:5, increase during fasting (Van Gilst *et al.* 2005b).

C. elegans severely deficient in PUFAs are viable, although they exhibit many growth, reproduction, and neurological defects (Watts and Browse 2002). The Δ12 desaturase *fat-2* mutants contain large amounts of oleic acid and only 1% PUFA. These mutants display many defects, including slow growth, very low brood size, and uncoordinated movement. The Δ6 desaturase *fat-3* mutants contain C18 PUFAs, but not C20 PUFAs. They grow better and display higher brood size than *fat-2* mutants, but show many defects compared to wild type (Lesa *et al.* 2003; Watts *et al.* 2003). The *fat-3* mutants have uncoordinated movement, which may be due to depleted synaptic vesicles and reduction of neurotransmitter release (Lesa *et al.* 2003). The *fat-3* mutants are defective in chemosensation, olfactory adaptation, touch sensitivity, and adaptation to alcohol (Kahn-Kirby *et al.* 2004; O'Halloran *et al.* 2009; Raabe *et al.* 2014; Vasquez *et al.* 2014). The *fat-4* and *fat-1* mutants contain different species of PUFAs and greatly different ratios of omega-6 and omega-3 species, although growth, movement, and reproduction are essentially normal

(Watts and Browse 2002). Therefore, many reproductive and neurological functions of PUFAs can be fulfilled by either omega-6 or omega-3 fatty acids. However, dietary omega-6 fatty acids, such as dihommogamma linolenic acid (DGLA, 20:3) adversely affect germ cell maintenance, leading to sterility above certain concentrations of dietary DGLA (Watts and Browse 2006; Webster *et al.* 2013). More details regarding the functions of PUFAs in *C. elegans* can be found in a recent review (Watts 2016).

Polyunsaturated fatty acids as precursors for signaling molecules

In response to signaling events, PUFAs are liberated from membranes by phospholipases and further metabolized to form powerful short-range signaling molecules, collectively termed eicosanoids. Eicosanoids are formed by several pathways that introduce molecular oxygen in highly stereo specific reactions. In mammals, eicosanoids are formed by cyclooxygenase, lipoxygenase, and cytochrome P450 (CYP) enzymes (Funk 2001; Spector and Kim 2015). While early papers noted that *C. elegans* do not contain homologs of cyclooxygenase enzymes that are responsible for synthesizing prostaglandins, several F-series prostaglandins have been identified in worm lipid extracts using mass spectrometry (Hoang *et al.* 2013). These F-series prostaglandins are synthesized from several C20PUFA precursors in the female germline and they function to attract sperm to oocytes (Kubagawa *et al.* 2006; Hoang *et al.* 2013). While the precise mechanism of prostaglandin formation has not been elucidated, it is likely that in both *C. elegans* and mammals, prostaglandins are synthesized using a cyclooxygenase-independent pathway (McKnight *et al.* 2014).

CYP enzymes produce eicosanoids from PUFAs by way of two pathways. One pathway uses ω-hydroxylation to convert PUFAs such as arachidonic acid to hydroxyeicosatetraenoic acids (HETEs), and another uses epoxygenase activity to generate epoxyeicosatrienoic acids (EETs); both pathways are active in *C. elegans* and the eicosanoid products of PUFAs have potent biological effects (Kulas *et al.* 2008). CYP-33E2 is expressed in the pharynx, and it produces EETs that are required for efficient pharyngeal pumping (Kosel *et al.* 2011). CYP-33E2 also acts on DGLA (20:3) and mediates the sterility that occurs after administration of dietary DGLA (Deline *et al.* 2015). Finally, the epoxygenase activity of CYP-13A12 is required for a behavior response to reoxygenation after oxygen deprivation (Ma *et al.* 2013; Keller *et al.* 2014).

Storage Lipids

Synthesis of neutral glycolipids

In animals, plants, and fungi, fatty acids are stored in the form of TAGs, which are efficient energy storage molecules due to their reduced state (Figure 1E). *De novo* synthesized or dietary fatty acids are esterified onto glycerol molecules to produce TAGs for storage. In addition, other nutrients, such as carbohydrates and amino acids, can be broken down into acetyl CoA for *de novo* fatty acid synthesis, and other metabolites can enter the glycerol-3-phosphate pathway to form diacylglycerols (DAG) and TAGs (Figure 3). Importantly, phospholipid and neutral lipid synthesis are linked, because both biosynthetic pathways share a common intermediate: DAG. *C. elegans* contains homologs of the glycerol-3 phosphate acyl transferase (GPAT) and the lysophosphatidic acid acyl transferase (LPAAT) families, which are involved in the transfer of acyl groups onto glycerol-3 phosphate and lysophosphatidic acid to synthesize phosphatidic acid (PA). Biochemical activity or substrate specificities of the many *C. elegans* GPAT and LPAAT homologs have not been determined. PA is then dephosphorylated by the lipin homolog LPIN-1 to generate DAG (Golden *et al.* 2009). TAGs are synthesized from DAGs by the activity of diacylglycerol acyl transferases (DGATs), one isoform of which is encoded by *dgat-2* (Xu *et al.* 2012). Fatty acyl-CoAs for TAG synthesis are either synthesized *de novo*, or obtained from the diet. The *E. coli* (OP50) strain does not synthesize or store TAGs; however, bacterial membranes are composed of phospholipids composed of fatty acids. These fatty acids are liberated from phospholipids during the digestion process, after which they are absorbed and are either directly incorporated into TAG or phospholipids, or further modified before incorporation into *C. elegans* lipids.

While digestion has not been actively studied in *C. elegans*, the intestine is acidic, and many genes encoding secreted lipases have been identified as intestinally expressed genes (McGhee 2007). Digestion begins with the disruption of bacteria in the pharynx, and further disruption of cells by secreted lysozymes. Because intact phospholipids cannot be imported across membranes, lipids are absorbed after dietary bacterial membrane lipids are acted on by secreted phospholipase enzymes that hydrolyze phospholipids into lysophospholipids and free fatty acids, which can then be imported across membranes into intestinal cells. Studies of the *pept-1* mutants, which are defective in the transport of dietary peptides into the intestine, reveal a relationship between the peptide transporter PEPT-1, the sodium proton exchanger NHX-2, and fatty acid intake, which, in PEPT-1 deficiency, leads to greatly increased fat accumulation (Spanier *et al.* 2009). Digestion and lipid assimilation are very efficient processes, given that, at the peak of egg laying, each *C. elegans* hermaphrodite consumes several million bacteria, and converts her entire body mass into embryos every day (McGhee 2007).

Lipid droplet and yolk lipoprotein structure

TAGs are the major component of lipid droplets, which accumulate in the intestine, epidermis, and germ line of *C. elegans*. Electron microscopy and lipidomic analysis of lipid droplets reveal that lipid droplets consist of a core of TAGs, surrounded by a monolayer of phospholipid and protein (Zhang *et al.* 2010b; Vrablik *et al.* 2015). TAGs are also a component of yolk, similar to mammalian lipoproteins,

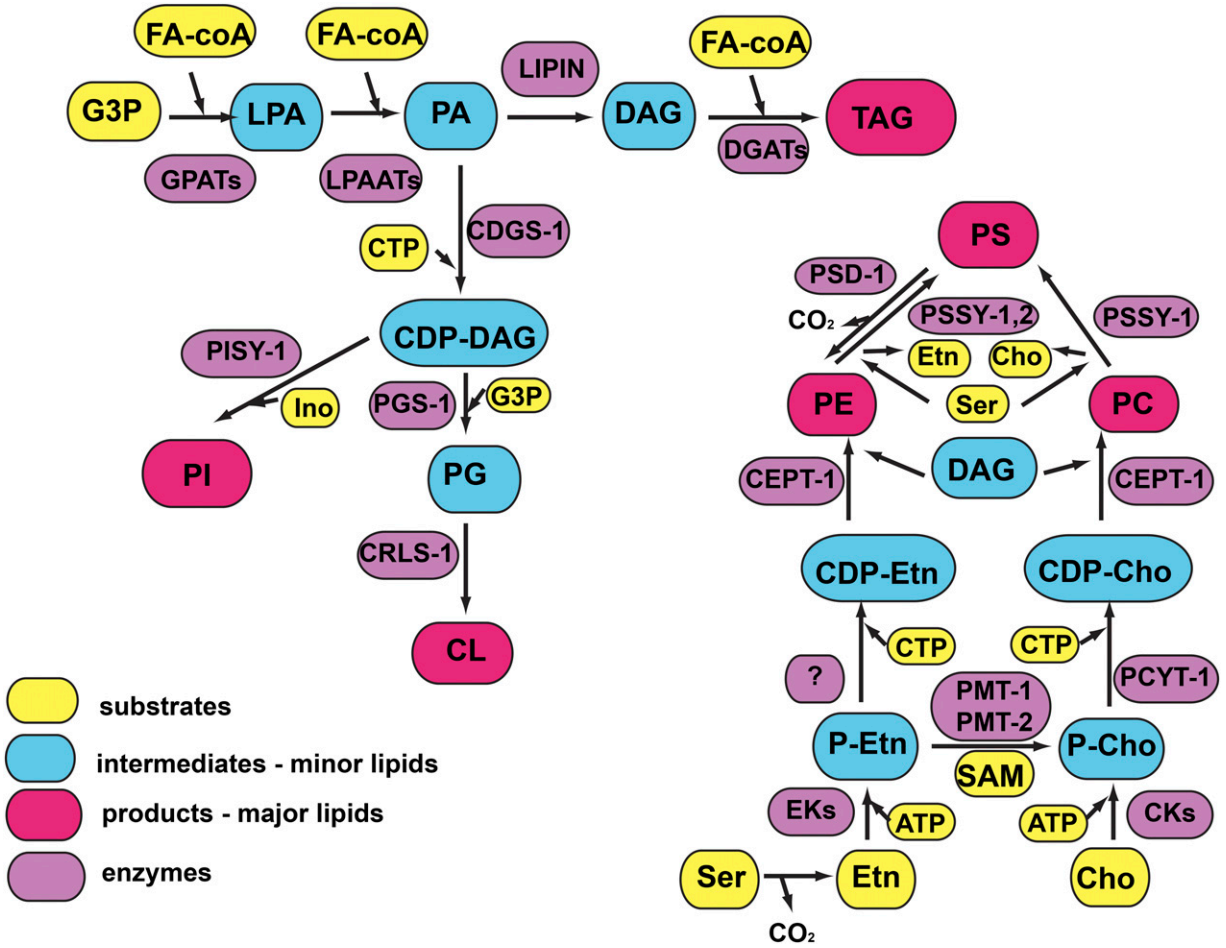

Figure 3 Biosynthesis of neutral lipids and glycerophospholipids. Substrates are depicted in yellow ovals, minor lipids and intermediates are depicted in blue ovals, major lipid products are depicted in red ovals, and enzymes are shown in purple ovals. G3P, glucose-3 phosphate; FA-coA, fatty acyl coenzymeA; Ino, inositol; Ser, serine; Etn, ethanolamine; SAM, s-adenosyl methionine; Cho, choline; CTP, cytidine triphosphate; LPA, lysophosphatidic acid; PA, phosphatidic acid; DAG, diacylglycerol; CDP-DAG, cytidine diphosphate diacylglycerol; PG, phosphatidylglycerol; CDP-Etn, cytidine diphosphate ethanolamine; CDP-Cho, cytidine diphosphate choline; P-Etn, phosphoethanolamine; P-Cho, phosphocholine; TAG, triacylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; CL, cardiolipin; PE, phosphatidylethanolamine; PC, phosphatidylcholine; GPAT, glycerol-3 phosphate acyl transferase; LPAAT, lysophosphatidic acid acyl transferase; DGAT, diacylglycerol acyl transferase; EK, ethanolamine kinase; CK, choline kinase.

which is synthesized in the intestine and transferred to developing oocytes during reproduction (Kimble and Sharrock 1983; Hall *et al.* 1999). The major protein components of yolk are the vitellogenin proteins YP170A, YP170B, YP115, and YP88, which are encoded by the genes *vit-1*, *-2*, *-3*, *-4*, *-5*, and *-6* and exist as two distinct lipoproteins complexes (Spieth and Blumenthal 1985; Heine and Blumenthal 1986; Sharrock *et al.* 1990). Yolk granules are estimated to be ~85% protein and 15% lipid by mass (Sharrock *et al.* 1990) and are ~0.5–1 μm in size. Yolk is synthesized in intestinal cells, exocytosed, and taken up by RME-2 receptor in oocytes, and also by coelomocytes (Grant and Hirsh 1999). Lipidomic analysis revealed that yolk contains relatively greater amounts of phospholipids than TAGs (Sharrock *et al.* 1990; Kubagawa *et al.* 2006). In contrast, lipid droplets contain a much higher relative amount of TAGs compared to phospholipids, and also are composed of much less protein compared to yolk granules (Vrablik *et al.* 2015). Lipid droplet are typically 0.5–2 μm in size, but can reach sizes up to 10 μm (Li *et al.* 2016). Neither yolk nor lipid droplets contain measurable amounts of cholesterol or cholesterol esters (Kubagawa *et al.* 2006; Vrablik *et al.* 2015). The relationship between the biosynthesis of yolk and lipid droplets is still unclear, and it is unknown how lipid droplets are transported to, or generated in, the germ line. Interestingly, vitellogenin proteins have been identified in *C. elegans* lipid droplet proteomes (Zhang *et al.* 2012; Vrablik *et al.* 2015), and a recent study reported that VIT-2 is involved in the transfer of dietary lipids to lipid droplets (Wang *et al.* 2016).

The major lipid droplet protein is PLIN-1, previously called MDT-28 (Zhang *et al.* 2012; Chughtai *et al.* 2015; Na *et al.* 2015; Vrablik *et al.* 2015). PLIN-1 is the closest *C. elegans* homolog of perlipins, which are a family of major structural lipid droplet proteins found in a wide variety of animals and fungi (Brasaemle 2007). When PLIN-1 is absent, F22F7.1, the homolog of the mammalian lipid droplet protein CGI-49,

increases in abundance on the surface of lipid droplets (Na *et al.* 2015). PLIN-1 is especially abundant in embryos, and embryos produced by *plin-1* RNAi contain lipid droplets that clump together, indicating the importance of PLIN-1 in stabilizing the structure of lipid droplets in embryos (Na *et al.* 2015; Vrablik *et al.* 2015). Other *C. elegans* lipid droplet-associated proteins include the ACS-22(FAT1P) and DGAT-2 complex, which is required for the generation of lipid droplets (Mak 2012; Xu *et al.* 2012), DHS-3 (Zhang *et al.* 2012; Na *et al.* 2015), ACS-4 (Vrablik *et al.* 2015), ATGL-1 (Zhang *et al.* 2010a), and LID-1 (Lee *et al.* 2014).

Several activities are important for the formation of lipid droplets and for the regulation of lipid droplet size. The FITM-2 protein, a member of the FIT family of conserved ER proteins required in yeast and mammals for the generation of lipid droplets at the ER, is also required for lipid droplet formation in *C. elegans* (Choudhary *et al.* 2015). The *fitm-2* mutants are lethal, implying that lipid droplet formation is essential. Lipid biosynthesis genes, including FAT-6 and FAT-7 are required for lipid droplet expansion, even in genetic backgrounds that normally promote increased fat stores (Shi *et al.* 2013). The atlastin GTPase is required for proper ER structure and lipid droplet size expansion (Klemm *et al.* 2013). The peroxisomal β-oxidation proteins MAOC-1, DHS-28, and DAF-22 are also required for the regulation of lipid droplet size (Zhang *et al.* 2010a). Mutants in these genes all accumulate large lipid droplets, indicating a requirement for the peroxisome in the utilization of fat from lipid droplets. Several recent genetic screens have identified a range of lipid droplet size regulators in embryos (Schmokel *et al.* 2016) and in adults, including several new proteins involved in lipid droplet fusion (Li *et al.* 2016).

Neutral lipid catabolism

To utilize fatty acids for energy, they must first be liberated from TAG molecules, and then broken down by β-oxidation, the sequential cleaving of two-carbon units to produce acetyl-CoA, which then enters the citric acid cycle to produce CO_2, NADH, and ATP. A reverse genetic screen of lipases identified ATGL-1, the homolog of mammalian adipocyte triglyceride lipase as the major lipase involved in liberating fatty acids from TAGs from lipid droplets during fasting (Lee *et al.* 2014). ATGL-1 is responsible for the rapid burning of fat in dauer larvae carrying mutations in AMP kinase, and thus participates in the rapid fat burning that contribute to the demise of AMP kinase-deficient dauer larvae (Narbonne and Roy 2009). The phosphorylation of ATGL-1 by AMP kinase generates 14-3-3 binding sites on ATGL-1, which leads to the dissociation of ATGL-1 from lipid droplets and eventual protesosomal degradation of ATGL-1 (Xie and Roy 2015a). A *C. elegans* homolog of the mammalian lipid droplet-associated protein CGI-58, called ABHD-5.2 in *C. elegans*, was shown to coactivate ATGL-1 by tethering it to lipid droplets (Xie and Roy 2015b). In addition, CGI-58/ABHD-5.2 acts independently of ATGL-1 to prevent fusion of lipid droplets, which might stem from changes in PUFA levels of the phospholipids surrounding lipid droplets (Xie and Roy 2015b).

Autophagy is the process of recycling of cellular material, including damaged proteins, mitochondria, peroxisomes, and lipid droplets to the lysosome for degradation. During fasting, the lysosome plays an important role in the early catabolic steps of lipid degradation (Settembre and Ballabio 2014). *C. elegans* contains at least three lysosomal lipases, LIPL-1, LIPL-3, and LIPL-4 (Wang *et al.* 2008; Lapierre *et al.* 2011; O'Rourke and Ruvkun 2013; Folick *et al.* 2015). LIPL-4 has been studied the most because of the link between autophagy, lysosomal lipase activity, and the extension of lifespan in germline-less nematodes (Wang *et al.* 2008; Lapierre *et al.* 2011). As described in *Dietary and endogenous synthesized monounsaturated fatty acids regulate fat accumulation and longevity*, Δ9 desaturases and their regulators NHR-80 and NHR-49 are required for the generation of lipid signals that promote longevity (Goudeau *et al.* 2011; Ratnappan *et al.* 2014). Thus, lipid synthesis and lipid catabolism are both required for increased longevity in animals lacking a germline. This may be explained by the recent findings that increased yolk lipoproteins are detrimental to longevity (Seah *et al.* 2016), and that, in constantly feeding worms, the lack of germline leads to energy imbalance in which greatly increased yolk accumulation occurs in intestinal cells and in pseudocoelomic spaces (Steinbaugh *et al.* 2015). The excess yolk has been proposed to trigger autophagy pathways regulated by HLH-30 to induce lipases required for the breakdown of excess lipids, while at the same time contributing to the production of lipid signals, such as oleyl ethanolamide (OEA), which is transported to the nucleus by way of the lysosomal lipid chaperone LBP-8, and serves as a ligand to activate NHR-80 and NHR-49 (Folick *et al.* 2015). Similar lipid signals derived from oleic acid could activate SKN-1, which, together with NHR-49, activate lipid catabolic pathways and other protective pathways leading to increased longevity (Ratnappan *et al.* 2014; Steinbaugh *et al.* 2015). In addition, the transcription factors DAF-16/FOXO and TCER-1 regulate lipid metabolism pathways in animals lacking a germline (Amrit *et al.* 2016). Interestingly, even though autophagy and lysosomal lipases are important components of lipid catabolism, well fed, fertile *C. elegans* mutants defective in autophagy processes actually have reduced, not increased, lipid accumulation compared to wild type (Lapierre *et al.* 2012). This indicates that autophagy is required for overall metabolic homeostasis in *C. elegans*.

β-*Oxidation of fatty acids*

Homologs of mitochondrial and peroxisomal genes encoding proteins involved in β-oxidation of fatty acids are present in *C. elegans* (Van Gilst *et al.* 2005b). The mechanism of the core β-oxidation cycle involves a dehydrogenation, hydration, dehydrogenation again, and a thiolytic cleavage, in which each cycle produces acetyl-CoA, NADH, and $FADH_2$, and an acyl group that is two carbons shorter than before. The four enzymes required include acyl-CoA dehydrogenase in the mitochondria or acyl-CoA oxidase in peroxisomes, enoyl-CoA hydratase, 3-hydroxylacyl-CoA dehydrogenase,

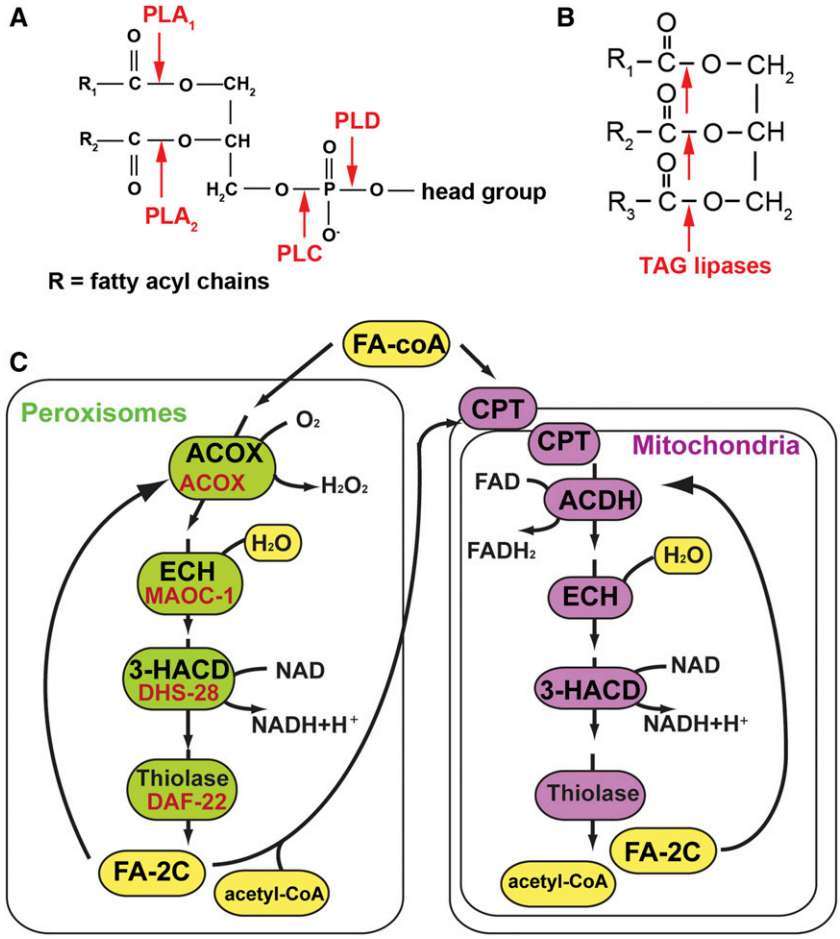

Figure 4 Lipid catabolism. (A) Location of phospholipase activity on a hypothetical phospholipid. (B) Location of TAG lipase cleavage on triacylglycerol. (C) Pathway of β-oxidation in peroxisomes and mitochondria. FA-coA, fatty acyl coenzymeA; CPT, carnitine palmitoyl transferase; ACOX, acyl coenzyme A oxidase; ECH, enoyl CoA hydratase; 3-HACD, 3-hydroxyacyl CoA dehydrogenase; FA-2C, a fatty acyl group two carbons shorter than when it started the cycle.

and 3-ketoacyl-CoA thiolase (Figure 4). Peroxisomal β-oxidation has been studied more in *C. elegans* than mitochondrial beta oxidation, because the peroxisomal enzymes are required for synthesis of ascarosides, signaling glycolipids based on the sugar ascarylose linked to short chain fatty acids [*Dideoxysugars* (*ascarosides*); Ludewig and Schroeder 2013]. For peroxisomal oxidation and ascaroside formation, the genes encoding the major activities of the pathway include various *acox* genes, *maoc-1*, *dhs-28*, and *daf-22* (Figure 4) (Jorgensen and Mango 2002; Butcher *et al.* 2009; von Reuss *et al.* 2012).

A β-oxidation-like pathway is used in *C. elegans* for the breakdown of propionate under conditions of limiting vitamin B12 (Watson *et al.* 2016). Propionate is a three-carbon short-chain fatty acid breakdown product of odd-chain fatty acids and branched chain amino acids that is normally catabolized by a carboxylation pathway that depends on vitamin B12. In the alternative pathway, the short-chain acyl-CoA dehydrogenase ACDH-1 converts propionyl-CoA to acrylyl-CoA, which is oxidized by the β-oxidation enzyme ECH-6, and further broken down into acetyl-CoA by the activities of HACH-1, HPHD-1, and ALH-8 (Watson *et al.* 2016).

In mammals, the peroxisome is involved in breaking down very long chain fatty acids, while mitochondrial oxidation breaks down long, medium, and short chain fatty acids. However, in plants, and in most fungi, fatty acid oxidation takes place solely in peroxisomes (Wanders and Waterham 2006). While the relative proportions of fat oxidation occurring in mitochondria or peroxisomes are not known in *C. elegans*, genetic screens point to the importance of peroxisomal oxidation for the breakdown of fats in large lipid droplets (Zhang *et al.* 2010a; Li *et al.* 2016). A biochemical assay for *C. elegans* β-oxidation has been developed (Elle *et al.* 2012). In addition to fat oxidation defects in AMP kinase mutants and *atgl-1* mutants, this assay has been used to demonstrate altered β-oxidation in *fat-6*;*fat-7* double mutants (Shi *et al.* 2013), *acs-3* mutants (Mullaney *et al.* 2010) and *daf-2* mutants (Elle *et al.* 2012). A functional role for β-oxidation has been shown using RNAi knockdown of β-oxidation genes to implicate increased fat oxidation as the mechanism of altered fat accumulation in various studies, including that of *cebp-2* mutants (Xu *et al.* 2015), *klf-3* mutants (J. Zhang *et al.* 2011), and in genetic backgrounds in which serotonin signaling is increased (Srinivasan *et al.* 2008).

Visualization of neutral lipids

Due to the human obesity epidemic and the serious diseases associated with obesity, there has been great interest in using *C. elegans* genetics to identify novel fat regulatory genes. Initial genetic screens used the lipophilic dye Nile Red in

nematodes to visualize fat accumulation in live animals (Ashrafi *et al.* 2003). Feeding of Nile Red was later determined to be problematic because Nile Red dye accumulates in lysosomal related organelles (LROs), also called gut granules, and this accumulation does not necessarily correspond to TAG levels in the worms (Schroeder *et al.* 2007; O'Rourke *et al.* 2009). Fixation of nematodes with isopropanol or paraformaldehyde and staining with Sudan Black, Nile Red, or Oil Red O produces visual patterns that are representative of biochemical determinations of fat levels (Brooks *et al.* 2009; O'Rourke *et al.* 2009; Pino *et al.* 2013; Wahlby *et al.* 2014). Lipid droplets in the intestine, germ line, and in epidermal tissues can be visualized with this technique. Another family of vital stains, BODIPY-labeled fatty acids, stains both lipid droplets and LROs in live nematodes, and has been successfully used to screen for mutants with supersized lipid droplets (Zhang *et al.* 2010a,b; Li *et al.* 2016). Lipids and fat depots have been visualized in label-free live animals using Coherent Anti-Raman Stokes (CARS) microscopy (Hellerer *et al.* 2007; Yen *et al.* 2010; Folick *et al.* 2011), whole-body imaging mass spectrometry (Hameed *et al.* 2015), and nonlinear microscopy imaging including second and third harmonic generation phenomena, which can detect ectopic fat deposits in muscle and neuronal tissues in aging worms (Mari *et al.* 2015; Palikaras *et al.* 2016).

Several strains expressing lipid droplet markers fused to GFP have been used to characterize lipid droplet size and abundance, although not every lipid droplet expresses the same lipid droplet proteins. Lipid droplets expressing DGAT-2 can be visualized in the intestine with the DGAT-2::GFP strain (Mak 2013), a subset of lipid droplets in the germline can be visualized with a ATGL-1::GFP and *Drosophila* PLIN-1::GFP, which localizes to *C. elegans* lipid droplets (Z. Liu *et al.* 2014).

Regulation of neutral lipid synthesis and breakdown

Lipid metabolism in *C. elegans* is regulated in response to environmental inputs, such as temperature and food availability, as well as by physiological state, including development, reproduction, and aging. Fat accumulation is altered depending on the bacterial strain used in culture (Brooks *et al.* 2009), food availability (Van Gilst *et al.* 2005b), temperature (Murray *et al.* 2007; Ma *et al.* 2015), iron (Wang *et al.* 2016), and various dietary treatments, such as supplementation of plant-derived extracts (Bhattacharya *et al.* 2013; El-Houri *et al.* 2014; Zheng *et al.* 2014; Ding *et al.* 2015; Gao *et al.* 2015; Peng *et al.* 2016; Sun *et al.* 2016).

The regulation of fat synthesis, desaturation, and lipid turnover is likely mediated at many levels, including transcription, translation, and protein stability. Of these processes, transcriptional regulation has been studied the most in *C. elegans*. The homolog of mammalian SREBP-1, SBP-1, regulates transcription of genes encoding proteins for fatty acid biosynthesis, especially *fat-5*, *fat-6*, *fat-7*, *elo-5*, *elo-6*, and genes encoding proteins required for phospholipid synthesis (see *Biosynthesis of phospholipids* below) (Kniazeva *et al.* 2004; Yang *et al.* 2006; Walker *et al.* 2011). SBP-1 null mutants arrest during early larval stages (McKay *et al.* 2003), while a hypomorphic allele, *sbp-1*(*ep79*), develops to adulthood, but displays slow growth, reduced reproduction, and low fat accumulation (Liang *et al.* 2010). Mutants defective in fat synthesis, including *sbp-1*, *fat-6*;*fat-7*, and *pod-2* fail to display normal satiety quiescence, in which worms stop feeding and reduce their activity—an activity analogous to sleep in mammals (You *et al.* 2008; Hyun *et al.* 2016). Thus, altered fat synthesis is detrimental to many aspects of *C. elegans* physiology.

Another major transcription factor regulating lipid metabolism in *C. elegans* is NHR-49 (Van Gilst *et al.* 2005a). This nuclear receptor is one of the large family of *C. elegans* HNF4-type NHRs, and it shows functional homology to mammalian PPARα—a mammalian regulator of lipid metabolism (Antebi 2015). NHR-49 regulates Δ9 desaturases, fat oxidation genes, sphingolipid synthesis genes, as well as nonlipid metabolism genes during normal development and also in response to fasting (Van Gilst *et al.* 2005b; Pathare *et al.* 2012). Both SBP-1 and NHR-49 associate with the mediator subunit MDT-15 to regulate transcription of lipid metabolism genes (Taubert *et al.* 2006; Yang *et al.* 2006). Gain-of-function alleles of *nhr-49* and *mdt-15*, together with genes required for PC synthesis, were isolated in a screen for suppressors of the cold-sensitive *paqr-2* strain, suggesting that the adiponectin homolog PAQR-2 regulates fatty acid desaturation during cold adaptation, in part by modulating PC abundance and SBP-1 activation (Svensk *et al.* 2013). In mutant strains carrying gain of function *nhr-49* alleles, many NHR-49 targets are upregulated, even those not involved in lipid metabolism (Lee *et al.* 2016). Another HNF4-type NHR, NHR-64, also regulates lipid metabolism, and RNAi of *nhr-64* partially suppresses the slow growth and low fat phenotypes of *sbp-1* and *fat-6*;*fat-7* mutants (Liang *et al.* 2010). In forward and reverse genetic screens, additional NHRs were identified as playing roles in regulating metabolic responses to various dietary inputs, as well as in regulating feeding behavior (Watson *et al.* 2013; Hyun *et al.* 2016). Hence, various nuclear receptors mediate a complex gene regulatory network regulating metabolic responses to the environment.

Fasting induces transcription of lipases, including fasting-induced lipases FIL-1 and FIL-2 (Jo *et al.* 2009), and the lysosomal lipase LIPL-1(O'Rourke and Ruvkun 2013). LIPL-1 is regulated by the bHLH transcription factor MLX-3, which represses *lipl-1* expression in the fed state, while, after food removal, another bHLH transcription factor HLH-30 activates expression of *lipl-1* and other lipases (O'Rourke and Ruvkun 2013). HLH-30 is also required in well-fed worms to limit ectopic lipid deposition in neuronal and muscle tissue in aging worms (Palikaras *et al.* 2016). Transcription of the adipocyte triglyceride lipase homolog *atgl-1* is repressed by the bHLH homolog HLH-11 in the presence of food, and then activated by NHR-76 in response to serotonin signaling (Noble *et al.* 2013; Srinivasan 2015).

Serotonin, also known as 5-HT, controls many food-related behaviors, including food intake, energy expenditure, locomotion,

and reproduction (Horvitz *et al.* 1982; Loer and Kenyon 1993; Waggoner *et al.* 1998; Sawin *et al.* 2000; Sze *et al.* 2000). Increasing serotonin signaling leads to rapid decrease of body fat as well as increased locomotion and feeding. However, increased feeding behavior and increased fat expenditure are regulated by independent neuronal circuits, in which the serotonin-gated chloride channel MOD-1 is involved in as the receptor required for loss of body fat, yet has no role in the behavioral responses to increased serotonin (Srinivasan *et al.* 2008). Three G-protein coupled serotonin receptors, SER-1, SER-5, and SER-7 are required for changes in feeding rates, but do not affect body fat composition (Srinivasan *et al.* 2008; Cunningham *et al.* 2012; Song and Avery 2012). For the regulation of body fat levels, serotonin and octopamine act together in a neural circuit to sustain body fat expenditure (Noble *et al.* 2013). A more detailed review of the neuronal control of fat accumulation is found in several recent publications (Lemieux and Ashrafi 2015; Srinivasan 2015).

A post-transcriptional regulator of fat accumulation was recently identified in a screen for genes required for survival during cold adaptation. The RNAse REGE-1 promotes the accumulation of fat by degrading ETS-4, a transcription factor that promotes the expression of fat catabolism genes (Habacher *et al.* 2016). Interestingly, ETS-4 also promotes expression of REGE-1, establishing an auto-regulatory loop for fine control of fat breakdown.

Two well-studied endocrine pathways required to promote reproductive growth under favorable nutrient conditions are the TGFβ/DAF-7 pathway and the insulin-like growth factor pathway, including the insulin-like growth factor receptor DAF-2. These developmental fate regulators are important in adult stage nematodes for body fat homeostasis, as reduction of function of *daf-2* or *daf-7* during adulthood leads to greatly increased fat accumulation (Kimura *et al.* 1997; Greer *et al.* 2008). DAF-7 is produced by a pair of ASI neurons and acts as a food sensor as well as a cell-nonautonomous regulator of fat synthesis and utilization (Greer *et al.* 2008). The DAF-2 receptor is expressed widely in the nervous system, and regulates fat via DAF-16/FOXO transcription factor activity in the intestine. The *daf-2* mutants accumulate large lipid droplets (Shi *et al.* 2013), and exhibit a higher rate of *de novo* fat synthesis than wild type (Perez and Van Gilst 2008).

Mechanistic target of rapamycin (mTOR) is a conserved serine/threonine kinase that acts as a nutrient sensor and promoter of growth. The mTOR kinase acts in two distinct complexes, TORC1, which consists of DAF-15/Raptor and TORC2, which consists of RICT-1/Rictor. Null mutants of *let-363*/mTOR and *daf-15*/Raptor arrest as L3 stage larvae (Long *et al.* 2002; Jia *et al.* 2004), while mutants in *rict-1*/Rictor display delayed growth rates, increased fat stores, and long lifespan (Jones *et al.* 2009; Soukas *et al.* 2009). The effects of TORC2 are mediated by SGK-1, a homolog of serum- and glucocorticoid-induced kinases. Recently it was reported that the *lin-4* and *let-7* microRNA program, which is best known for acting in the epidermis to control developmental timing, also signals to the intestine through SGK-1 and TORC2 to coordinate yolk protein synthesis upon the initiation of reproduction (Dowen *et al.* 2016).

The TORC1 complex is regulated by nutrient intake, when nutrients are scarce, AAK-1/AMP kinase acts on the TSC1 complex to inhibit the conversion of GTPase activating protein RHEB to RHEB-GTP, leading to repression of TORC1 complex activity. While the components of this signaling pathway are not well defined in *C. elegans*, a recent study revealed that ATX-2, a homolog of human Ataxin-2, acts as a TORC1 repressor, and *atx-2* mutants exhibit increased body size, cell size, and fat accumulation (Bar *et al.* 2016). A downstream target of the TORC1 complex is the S6 kinase, called RSKS-1 in *C. elegans*. The S6 kinase regulates translation efficiency by phosphorylating ribosomal protein S6 and other proteins. *C. elegans rsks-1* mutants grow slowly, have a reduced brood size and a smaller pool of germline progenitor cells (Korta *et al.* 2012). The *rsks-1* mutant also have increased fat accumulation compared to wild type (Shi *et al.* 2013). This reduced progenitor pool is also observed in nutrient sensing-defective, high fat accumulating *daf-2* and *daf-7* mutants (Michaelson *et al.* 2010; Dalfo *et al.* 2012). Thus, in these three strains, as well as others, such as *glp-1* mutants, there is often a correlation between increased fat accumulation and reduced reproductive output, and often these strains exhibit lifespan extension as well (Lemieux and Ashrafi 2016).

Membrane Lipids

In *C. elegans*, membranes are composed of glycerophospholipids, sphingolipids, and very small amounts of cholesterol. Glycerophospholipids, hereafter called phospholipids, make up the bulk of membrane lipids, with PC and phosphatidylethanolamine (PE) being the most abundant (Satouchi *et al.* 1993) (Figure 1C).

Biosynthesis of phospholipids

Phospholipids are synthesized from several pathways including the CDP-DAG pathway and the Kennedy pathway (Figure 3). Both pathways begin with the addition of two acyl groups by acyl-transferase enzymes (GPAT and LPAAT homologs) to glycerol-3 phosphate (G3P), generating PA—a central metabolite in both neutral and phospholipid synthesis. Before transfer, the acyl groups must first be activated to acyl-CoAs, a reaction that is catalyzed by ACS enzymes. *C. elegans* has 23 ACS homologs, which potentially activate fatty acids for use in a variety of processes, including lipid synthesis, β-oxidation, protein acylation, and fatty acyl transport across membranes (Watkins *et al.* 2007). Several *C. elegans* ACS proteins that have been biochemically assayed, including ACS-1, which is required for mmBCFA synthesis (Kniazeva *et al.* 2008), and ACS-20 and ACS-22, which are FAT1p homologs required for the incorporation of C26:0 in sphingomyelin (Kage-Nakadai *et al.* 2010). Recently, ACS-4 was shown to have protein myristolation activity, and to regulate fertility by translating fatty acid levels into regulatory cues

affecting the germline sex-determination pathway (Tang and Han 2017). Other enzymes involved in phospholipid synthesis include the two acyl-transferase activities that contribute to PA synthesis from G3P, which are likely encoded by one or more of the 14 acyl-CoA ligases (ACL) enzymes that are homologs of GPAT and LPAAT. While the GPAT and LPAAT activities involved in PA biosynthesis have not been characterized in *C. elegans*, four ACL enzymes required for other activities have been characterized. These include ACL-7, the mammalian GNPAT homolog that acts in the peroxisome for the first steps of the synthesis of ether-linked lipids (see *Synthesis of ether-linked PE species*) (Shi *et al.* 2016) and ACL-8, ACL-9, and ACL-10/BUS-18, which, together with the phospholipase *ipla-1*, are responsible for the remodeling of PI such that stearic acid is transferred to the sn1 position (Imae *et al.* 2010).

In the CDP-DAG pathway, PA and CTP are converted to CDP-DAG by CDP-DAG synthase, encoded by the *cdgs-1* gene. The CDP-DAG pathway permits synthesis of phosphatidyl inositol (PI) and phosphatidyl glycerol (PG) by the addition of the inositol (*pisy-1*) and glycerol-3 phosphate (*pgs-1* to CDP-DAG (Figure 3). The mitochondrial lipid cardiolipin (CL) is synthesized from two PG molecules by cardiolipin synthase, CRLS-1 (Sakamoto *et al.* 2012).

The Kennedy pathway also operates in *C. elegans*, in which choline and ethanolamine are phosphorylated by the activities of choline- and ethanolamine-kinases (encoded by *cka-1*, *cka-2*, *ckb-1*, *ckb-2*, *ckb-3*, *ckb-4*), then activated by CTP-phosphocholine cytidylyl transferase (*pcyt-1*) (Walker *et al.* 2011) and CTP-phosophoethanolamine cytidylyl transferase. The activated CDP-choline and CDP-ethanolamine then react with DAG to form PE and PC, with the enzyme CEPT-1. In many organisms, PC can by synthesized from PE by three sequential methylation reactions, using s-adenysyl methionine (SAM) as a substrate and methyl transferase enzymes (Brendza *et al.* 2007; Walker *et al.* 2011). Interestingly, while most animals can convert PC from PE using PE methyl transferases, *C. elegans* perform this methylation in a manner similar to plants, using phosphoethanolamine as a substrate and the phosphoethanolamine methyl transferase enzymes PMT-1 and PMT-2 (Brendza *et al.* 2007) (Figure 3).

Phosphatidylserine (PS) is formed from the exchange of serine with choline or ethanolamine moieties of PC or PE by the PS synthase enzymes 1 and 2 (PSSY). In mammals, PTDSS1 (homolog of PSSY-1) can use either PE or PC as a substrate, while PTDSS2 (homolog of PSSY-2) only uses PE. Substrate specificities of PSSY-1 and PSSY-2 have not been determined in *C. elegans*. PS can be converted back to PE with the activity of PS decarboxylase (PSD-1) (Figure 3).

The importance of the phosphocholine methylation pathway for PC synthesis is apparent when observing *sams-1* mutants. These mutants exhibit low levels of PC, which can be rescued by dietary choline (Walker *et al.* 2011). In the absence of dietary choline, the *sams-1* worms exhibit large lipid droplets, and high PE and TAG levels, which may be due to lipid homeostasis disruption that occurs when DAG precursors are not used for PC synthesis, and instead are diverted into the neutral lipid synthesis pathway (Li *et al.* 2011; Walker *et al.* 2011; Ehmke *et al.* 2014). During a moderate, post embryonic dietary restriction regime, lipid stores increase, and reproduction slows, even though the restricted worms produce normal numbers of progeny over their lifetime (Palgunow *et al.* 2012; Miersch and Doring 2013). Interestingly, dietary choline abrogates this increased lipid droplet size and TAG levels, indicating that methyl donors and one carbon metabolites are key factors for normal lipid homeostasis (Ehmke *et al.* 2014).

Membrane asymmetry and remodeling

In animals, membrane lipid composition varies depending on cell and organelle type, but, in *C. elegans*, phospholipid analysis has only been performed using whole worms because of the difficulty in isolating enough pure tissues for lipid analysis. It is likely that the distribution of various phospholipids is not uniform throughout the membranes of intercellular organelles or among distinct tissues. For example, based on lipid analysis of other animals, cardiolipin is mainly a component of mitochondrial membranes, while sphingomyelin is mainly localized to the outer leaflet of plasma membranes.

Class IV P-type ATPases translocate PS or PE between two leaflets of membranes, maintaining a higher concentration of PE and PS on the inner leaflet of the plasma membrane (Folmer *et al.* 2009). *C. elegans* encodes six genes predicted to encode class IV P-type ATPases: *tat-1*–*tat-6*. Mutants lacking *tat-1* show increased PS staining on the outer surface of germ cells, indicating that TAT-1 functions to maintain PS in the inner membrane leaflet (Darland-Ransom *et al.* 2008). The externalization of PS serves as an engulfment signal during the conserved process of apoptosis, and *tat-1* mutants were found to exhibit a random loss of neurons and muscle cells that was dependent on the PS-dependent phagocyte recognizing receptor PSR-1 (Darland-Ransom *et al.* 2008). Furthermore, TAT-1 and its chaperone CHAT-1 are required for proper membrane tubule formation, and, in their absence, endocytic sorting and recycling are defective (Chen *et al.* 2010). In the embryo, *tat-5* mutants release excessive extracellular vesicles, leading to disruptions in cell adhesion and morphogenesis during embryogenesis (Wehman *et al.* 2011). Staining embryos with duramycin, which binds to PE, revealed excessive PE on the outer membrane surface of *tat-5* mutant embryos, demonstrating a link between membrane lipid asymmetry and extracellular vesicle formation (Wehman *et al.* 2011).

The requirement for regulated phospholipid composition within the nuclear envelope was demonstrated in studies of *C. elegans* mutants defective in the PA phosphatase LIPIN (*lpin-1* mutants) as well as mutant strains of the nuclear envelope-localized LIPIN regulator CTDNEP1 (*cnep-1* mutants). In both mutant strains, the nuclear envelope fails to disassemble during mitosis (Golden *et al.* 2009; Gorjanacz and Mattaj 2009; Bahmanyar *et al.* 2014). Studies of the *cnep-1* mutants showed that ectopic ER sheets formed around the nuclear

envelope, preventing breakdown of the nuclear membrane during mitosis (Bahmanyar *et al.* 2014). The formation of the ectopic ER sheets correlated with higher levels of PI in the *cnep-1* mutants, and both PI levels and the ectopic sheet formation were rescued by RNAi inhibition of *pisy-1* or *cdgs-1*, which reduced PI synthesis (Bahmanyar *et al.* 2014). These studies demonstrate that spatial regulation of membrane phospholipid composition is critical for key cellular and developmental events.

The various phospholipids have distinct fatty acyl compositions (Table 1). It is likely significant fatty acyl remodeling is occurring to maintain the specific composition. For example, cardiolipin PUFA composition is unique, and, unlike other phospholipids, contains almost no Δ5 desaturated fatty acids (Hou *et al.* 2014) (Table 1). Adding fatty acyl groups to lysophospholipids—an activity important for membrane remodeling—uses membrane bound o-acyltransferases, abbreviated MBOA. *C. elegans* encodes seven *mboa* genes, and the biochemical activities of two MBOAs have been characterized. MBOA-6 has been shown to incorporate PUFAs into PC, PS, and PE (Matsuda *et al.* 2008). MBOA-7 incorporates long chain PUFA specifically into PI (Lee *et al.* 2008), and, as such, plays an important role in phosphoinositide signaling (Lee *et al.* 2012).

Synthesis of ether-linked PE species

Ether lipids are glycerolipids in which one of the fatty acyl groups, typically a saturated fatty acid, is linked to the sn1 position of glycerol backbone by an ether bond, rather than by the usual ester bond. Ether lipids can be in the alkylacyl form, or the alkenylacyl, which contain a double bond adjacent to the oxygen, and are also known as plasmalogens (Figure 1D). In *C. elegans*, ether-linked lipids are found mainly in PE, with very small amounts in PC (Satouchi *et al.* 1993; Drechsler *et al.* 2016; Shi *et al.* 2016); ~20% of PE species contain an alkylacyl bond, and 14% contain an alkenyl bond (Satouchi *et al.* 1993).

Ether-linked lipids are unusual in that their synthesis begins in the peroxisome (Brites *et al.* 2004). In the peroxisome, the acyl transferase ACL-7 transfers an acyl group to dihydroxyacetone phosphate, creating 1-acyl-DHAP. The ether-linkage is formed by the ADS-1 enzyme, which catalyzes the synthesis of 1-alkylacyl DHAP by using a reduced fatty alcohol, rather than a fatty acyl, as a substrate. The reduced fatty alcohol is produced by the fatty alcohol reductase FARD-1. Mutants in *acl-7*, *ads-1*, and *fard-1* lack the ability to synthesize ether-linked lipids (Shi *et al.* 2016). Analysis of the ether-lipid deficient mutants demonstrate that ether lipids are required for optimal reproduction, lifespan, survival at cold temperatures, and resistance to oxidative stress (Drechsler *et al.* 2016; Shi *et al.* 2016). All of the *C. elegans* ether-lipid deficient strains accumulate high levels of 18:0 in all lipids, presumably to compensate for the structural properties of ether-linked lipids, which are predicted to contribute to membrane rigidity.

The activity of AGMO-1, alkyglycerol monooxygenase, is required to break down ether-linked lipids (Watschinger *et al.* 2010). The *C. elegans agmo-1* gene is expressed in the epidermis, and *agmo-1* mutants, along with mutant strains lacking the ability to synthesize tetrahydrobiopterin, an AGMO-1 cofactor, have a fragile cuticle and are sensitive to exogenous chemicals (Loer *et al.* 2015). This indicates that oxidation of ether lipids is an important process involved in the formation of the nematode cuticle.

Phospholipid catabolism and signaling

Both synthesis and degradation of phospholipids occurs constantly, as membrane are consumed for cellular processes such as intracellular trafficking, organelle biogenesis, β-oxidation, and cellular repair. Phospholipid turnover has been measured in *C. elegans* using a ^{15}N incorporation assay analyzed by HPLC-MS/MS (Dancy *et al.* 2015). For this study, 210 distinct PC and PE species were followed, and the authors found that ~2% of phospholipid species are turned over every hour, with PE species turning over at rates higher than PC species (Dancy *et al.* 2015). The rates of fatty acyl alterations in phospholipids are even higher; thus, the majority of *C. elegans* lipids turnover daily (Dancy *et al.* 2015).

Fatty acyl groups are liberated from phospholipids by the activities of phospholipase enzymes (Figure 4A). There are five types of phospholipases that are defined by the position in a phospholipid in which they cleave. Phospholipases A_1 and A_2 cleave off a fatty acyl group at the sn-1 and sn-2 positions of phospholipids, respectively. Phospholipiase B enzymes possess both A_1 and A_2 activity. Phospholipase C enzymes cleave at the at the phosphate group, creating DAG and a phosphorylated headgroup, while Phospholipase D enzymes cleave on the other side of the phosphate group, creating PA and an alcohol. Phospholipases that are secreted into the lumen of the intestine aid in digestion of bacterial membranes, allowing for the uptake of bacterial fatty acids and lysophospholipids. Intracellular phospholipases play important roles in signaling, creating powerful short-range signaling molecules such as DAG, phosphoinositides, fatty acyl amides, and eicosanoids. Each family of phospholipases is represented by multiple gene products in *C. elegans*, and most phospholipase activities and substrate specificities have not been experimentally determined. In addition to their role in signaling, phospholipases also contribute to membrane maintenance by removing fatty acyl groups to allow acyl transferase enzymes to insert specific fatty acyl species for membrane remodeling.

C. elegans contains multiple gene encoding phospholipase A_2 homologs, and the group VIA, calcium-independent phospholipase A_2 homologs have been identified by sequence similarity to the human gene (Morrison *et al.* 2012). The *C. elegans* IPLA-1 was expressed and purified for biochemical studies examining its conformation, complex formation, and substrate specificity, and it was found to bind to many species of acidic phospholipids, including PA, PG, PS, CL, and phosphorylated derivatives of PI (Morrison *et al.* 2012). Knockdown of the six homologs showed that IPLA-2 is necessary for optimal progeny production (Morrison *et al.* 2012).

A study of the roles of lysosomes in stress responses, including short-term fasting, identified IPLA-2 as a target of NHR-49 in attenuating the overaccumulation of lysosomal lipids during fasting (Huang *et al.* 2014).

Among the best studied phospholipases are the phospholipase C family, which produces three distinct signaling molecules from PI: DAG, inositol 1,4,5-trisphosphate (IP_3), and phosphatidylinositol 4,5 bisphosphate (PIP_2) (Putney and Tomita 2012). Phospholipase C activity is regulated by calcium, and all three of the lipid signaling molecules regulate ion channels. *C. elegans* encodes at least five active PLC isoforms belonging to four PLC families: *plc-2* and *egl-8* (PLC-β), *plc-3* (PLC-γ), *plc-4* (PLC-δ), and *plc-1* (PLC-ε) (Gower *et al.* 2005). These PLC activities are important for a range of reproductive, neurological, and host defense processes (Gower *et al.* 2005; Vazquez-Manrique *et al.* 2008; Ziegler *et al.* 2009; Iwasa *et al.* 2010; Kawli *et al.* 2010; Xing and Strange 2010; Xu and Chisholm 2011; Kovacevic *et al.* 2013; Yu *et al.* 2013; Chun *et al.* 2015; Najibi *et al.* 2016; C. Zhang *et al.* 2016).

Phospholipase D activity produces N-acyl amides, lipid signaling molecules that include endogenous ligands of mammalian cannabinoid receptors (De Petrocellis *et al.* 2000). In *C. elegans*, at least six N-acetylethanolamines (NEAs) are generated from PE molecules by the phospholipase D isoforms NAPE-1 and NAPE-2 (Lucanic *et al.* 2011; Harrison *et al.* 2014). NEAs promote larval development, and are present at very low levels in starved L1 or dauer larvae. Dietary EPEA, derived from PE containing C20:5, rescues constitutive dauer formation in chemosensory mutants, TGFβ mutants and insulin signaling mutants required for food sensation and promotion of reproductive growth (Lucanic *et al.* 2011). Low levels of NEAs present in dietary restricted nematodes promote long lifespan, and dietary NEAs obliterate the lifespan extension (Lucanic *et al.* 2011). NAEs are degraded by fatty acid amide hydrolase FAAH-1, and overexpression of *faah-1* causes a developmental delay (Lucanic *et al.* 2011). OEA levels are affected by the lysosomal lipase LIPL-4, a lipase linked to longevity of germline deficient nematodes (Wang *et al.* 2008; Lapierre *et al.* 2012; Folick *et al.* 2015). OEA binds to LBP-8, a lipid chaperone required for lysosomal signaling to the nucleus (Folick *et al.* 2015). The nuclear OEA then binds to NHR-80, which acts with NHR-49 to regulate fatty acid desaturation and promote longevity (Folick *et al.* 2015).

Sphingolipid biosynthesis and function

Sphingolipids differ from glycerophospholipids in that they contain a sphingoid base, which is a long-chain aliphatic amine (Figure 1F). In most animals, the sphingoid base is typically the saturated straight chain of 18 carbons containing a double bond at C4, hydroxyl groups at C1 and C3, and an amino group at C2. In *C. elegans*, the long-chain sphingoid base is derived from the branched chain fatty acid C15:iso, which condenses with serine to form a d17:iso-sphinganine (Chitwood *et al.* 1995; H. Zhang *et al.* 2011). The condensation reaction uses the enzyme serine palmitoyl transferase, forming 3-keto sphinganine, which is reduced by the sphinganine reducatase and NADPH to form sphinganine (Figure 5). The serine palmitoyl transferase homologs are encoded by three genes in *C. elegans*, *sptl-1*, *sptl-2*, and *sptl-3*. RNAi of *sptl-1* results in L1 arrest with intestinal lumen polarity defects that resulted in L1 lethality (H. Zhang *et al.* 2011). This L1 lethality can be rescued by dietary supplementation of d17iso-sphinganine (Zhu *et al.* 2013).

The simplest sphingolipid, ceramide, consists of a fatty acid linked to the amine group of the sphinganine base (Figure 1F). In *C. elegans*, the amino linked fatty acids are primarily straight-chained saturated or 2-hydroxylated fatty acids of length C18–C26 (Chitwood *et al.* 1995; H. Zhang *et al.* 2011; Cutler *et al.* 2014). The long chain saturated or hydroxylated fatty acyl-CoA is added to the 17iso-sphinganine by ceramide synthase enzymes encoded by the *hyl-1*, *hyl-2*, and *lagr-1* genes. This forms d17iso-dihydroceramide, which is then desaturated by dihydroceramide desaturases, encoded by *ttm-5* and F33D4.4, to form ceramide. In *C. elegans*, in addition to its role as a structural membrane lipid, *de novo* ceramide synthesis is required to regulate apoptosis of germ cells after radiation damage (Deng *et al.* 2008), as well as for surveillance of mitochondrial function after genetic or drug-induced treatments that disable mitochondria (Y. Liu *et al.* 2014).

The ceramide synthases HYL-1 and HYL-2 transfer distinct acyl chains to the d17iso sphinganine. HYL-1 is specific for C24–C26 acyl chains, while HYL-2 preferentially catalyzes the reaction with C20–C22 acyl chains (Menuz *et al.* 2009). Mutants in *hyl-1* and *hyl-2* have opposing phenotypes, *hyl-1* mutants are resistant to anoxia, while *hyl-2* mutants are more sensitive than wild type, indicating that specific ceramides produced by HYL-2 confer resistance to anoxia (Menuz *et al.* 2009). The hypoxia sensitivity of *hyl-2* mutants can be suppressed by reduction of CYP-25A1 or CYP-33C8 (Ladage *et al.* 2016). Similarly, *hyl-2* mutants have short lifespans, while a double mutant of *hyl-1*;*lagr-1* produced worms with extended lifespan (Mosbech *et al.* 2013). Interestingly, the long lifespan of *hyl-1*;*lagr-1* double mutants can be suppressed by *sphk-1* RNAi, which reduces the activity of sphingosine kinase, which acts in the sphingolipid degradation pathway (Mosbech *et al.* 2013) (Figure 5). This provides evidence that specific sphingolipid species have opposing roles in lifespan determination.

Ceramides are modified to form more complex sphingolipids by the addition of phosphate or carbohydrate groups to form sphingomyelins, cerebrosides, or gangliosides (Figure 1F). Sphingomyelin is synthesized by the addition of a phosphatidylcholine group to C1 of ceramide, using sphingomyelin synthases encoded by *sms-1*, *sms-2*, *sms-3* (Huitema *et al.* 2004) (Figure 5). Sphingomyelins are the most abundant group of sphingolipids. In mammals, these lipids are typically localized to the outer leaflet of plasma membranes, and often are components of lipid microdomains that form a distinct structure for membrane-bound signal transduction proteins. The ACS-20 and ACS-22 activities are required for the addition of long chain fatty acyl groups to sphingomyelin, and

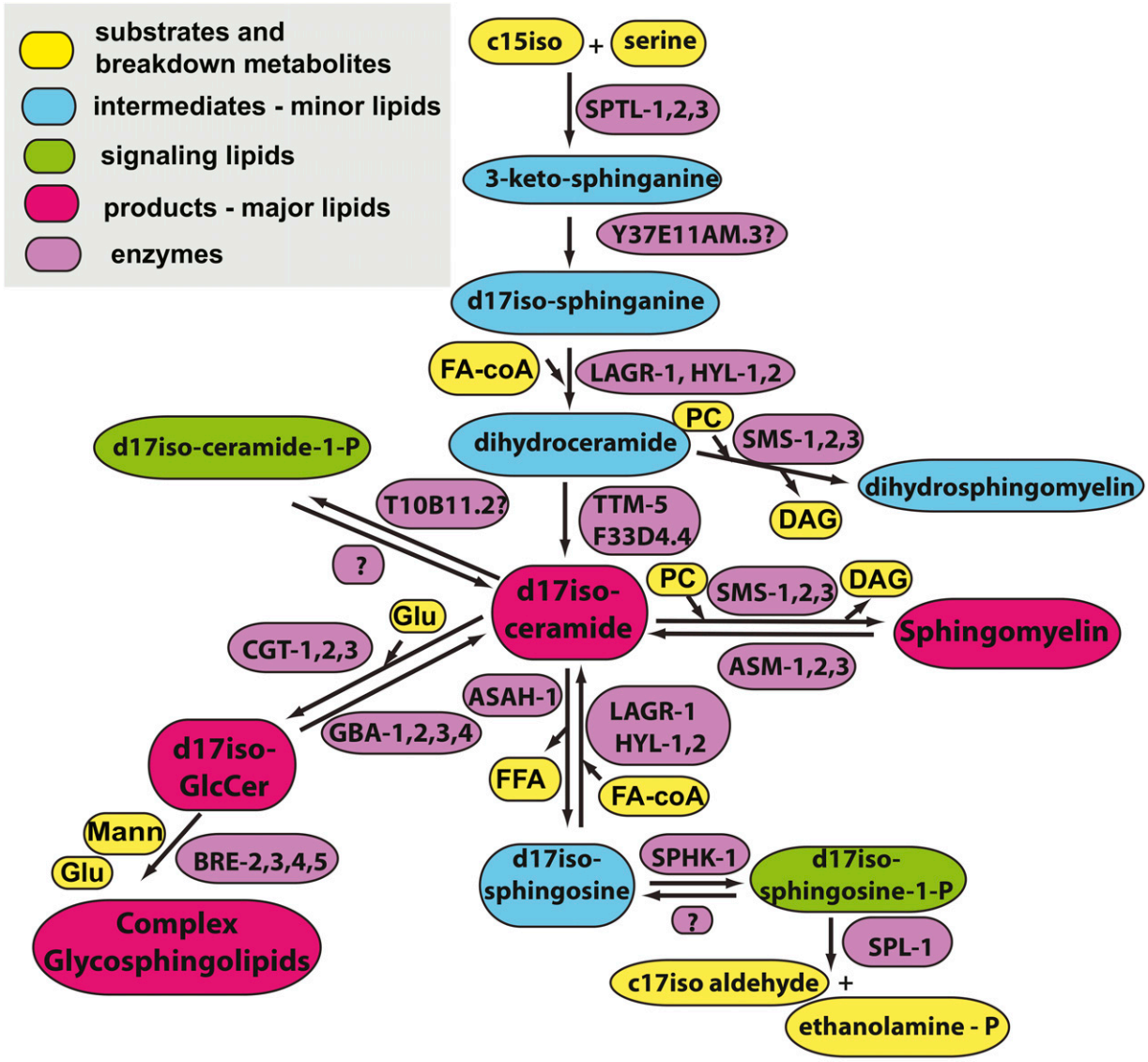

Figure 5 Sphingolipid synthesis and breakdown. Yellow ovals depict substrates and breakdown metabolites, blue ovals depict minor lipids and intermediates, green ovals depict signaling lipids, red ovals depict major lipid products, and purple ovals depict enzymes. C15iso, 13-methyltetradecanoic acid; FA-coA, fatty acyl coenzymeA; Glu, glucose; Mann, mannose; FFA, free fatty acid; PC, phosphatidylcholine; DAG, diacylglycerol.

acs-20 and *acs-20*;*acs-22* double mutants exhibit surface barrier defects, suggesting that sphingomyelins are components of the nematode cuticle (Kage-Nakadai *et al.* 2010).

Carbohydrate groups are added to ceramide by the action of three ceramide glucosyl transferases, CGT-1, CGT-2, and CGT-3 (Marza *et al.* 2009). Single mutants of *cgt-1*, *cgt-2* show no discernable phenotypes; however, *cgt-3* mutants have greatly reduced brood size and early embryo defects, and *cgt-3*;*cgt-1* double mutant has growth arrest as L1 (Nomura *et al.* 2011). Indeed, RNAi of *cgt-1* and *cgt-3* leads to aberrant lumen formation and L1 arrest, as does RNAi of *pod-2*, *let-767*, *acs-1*, *elo-3*, and *hpo-8*, all of which are involved in the synthesis and elongation of saturated fatty acids (H. Zhang *et al.* 2011). This systematic analysis of L1 intestinal lumen defects led to the identification of d17iso glucosylceramide (d17iso-GlcCer) as the sphingolipid required for proper polarity of intestinal cells and lumen formation in L1 larvae (H. Zhang *et al.* 2011; Zhu *et al.* 2013).

An interesting relationship between d17iso-GlcCer and the TORC1 signaling pathway was identified by an unbiased genetic screen in which the requirements for d17iso-GlcCer in larval development were ameliorated by a reduction of function mutation in *nplr-3*, which encodes a homolog of a NPR3—a nitrogen permease regulator that acts as a negative regulator of the TORC1 pathway (Zhu *et al.* 2013). Furthermore, hyperactivation of TORC1 also bypasses the need for mmBCFA and d17iso-GlcCer synthesis, leading to the idea that d17iso-GlcCer is required for TORC1 activation, which leads to proper L1 development (Zhu *et al.* 2013). Furthermore, the NPR-3/TORC1 signaling pathway acts

downstream of the d17iso-GlcCer pathway to regulate foraging behavior, which depends on the proper differentiation of sensory neurons in the L1 larval stage, and on proper food-seeking locomotion behavior in the adult (Kniazeva *et al.* 2015).

Finally, d17iso GlcCer can be further glycosylated by the β-mannosyl transferase BRE-3 and the glycosyltrasferases BRE-2, BRE-4, and BRE-5 (Griffitts *et al.* 2001, 2003, 2005). An array of complex glycosphingolipids can be formed through these activities. These glycosylated sphingolipids are produced by nematodes and arthropods, but not by mammals, and are targets of *Bacillus thuringiensis* toxins (Griffitts *et al.* 2001, 2003, 2005).

Breakdown of sphingolipids and ceramide signaling

The breakdown of plasma membrane sphingomyelins generates ceramides that act as signaling molecules in response to environmental stresses, such as heat shock, infection, radiation, or chemotherapy (Schuchman 2016). *C. elegans* contains acidic, but not neutral, sphingomyelinase activity (Lin *et al.* 1998). The acid sphingomyelinases are encoded by the *asm-1*, *asm-2*, and *asm-3* genes. Ceramides can diffuse through the membrane and activate various stress pathways, such as the JNK and ERK pathways that promote apoptosis and inflammation.

Ceramide is degraded through an acid ceramidase, encoded by *asah-1*, which deacylates to 17iso-sphingosine, which is subsequently phosphorylated by SPHK-1 sphingosine kinase to 17iso-sphingosine-1-phosphate (Zhang *et al.* 2015). This can be further degraded by sphingosine-1-phosphate lyase (*spl-1*), yielding ethanolamine phosphate and a C17iso aldehyde, which can be utilized for production of various other lipids. The *spl-1* gene is expressed in intestinal cells after hatching throughout larval development and adulthood (Mendel *et al.* 2003). Knockdown of *spl-1* by RNAi leads to severe defects in intestinal and reproductive function, demonstrating that the breakdown of sphongosine-1 phosphate is essential in *C. elegans* (Mendel *et al.* 2003).

Sterol and Prenol Lipids

The mevalonate pathway

Sterol and prenol lipids differ from the acyl-based lipids described above, in that the basic units are the five-carbon branched isoprene groups, isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Figure 1B). In plants and animals, these precursors are used to synthesize a large, diverse class of lipids including cholesterol, vitamin D, dolichol, coenzyme Q, and steroid hormones. In *C. elegans*, RNAi of genes encoding the enzymes of the mevalonate pathway leading to IPP and DMAPP result in embryo lethality, indicating that the synthesis of isoprenenoid precursors is essential (Kamath *et al.* 2003; Rauthan and Pilon 2011). These precursors are the substrates for various biosynthetic pathways that lead to the synthesis of coenzyme Q, dolichol, isopenteneyl pyrophosphate tRNAs, and prenylated proteins (Figure 6). Unlike mammals, *C. elegans* does not synthesize cholesterol and other steroids using the mevalonate pathway, because it lacks squalene synthase activity (Vinci *et al.* 2008). Details of the genes, enzymes, and biochemical reactions of the mevalonate pathway in *C. elegans* have been described in an extensive review (Rauthan and Pilon 2011).

The rate-limiting step in the mevalonate pathway is HMG-CoA reductase, the enzyme that is targeted by statin drugs, which are widely used to treat high cholesterol in humans. Statins induce embryo lethality and induction of ER unfolded protein response (UPR^{ER}) in *C. elegans* (Morck *et al.* 2009). A mutant in the HMG-CoA reductase, *hmgr-1*, shows phenotypes corresponding to those of the statin-treated *C. elegans*. The embryonic lethality and UPR^{ER} phenotypes of the *hmgr-1* mutant can be rescued with dietary mevalonate, which enabled the establishment of mevalonate levels required for various biological processes (Ranji *et al.* 2014). Reproductive processes require the highest levels of mevalonate, while rescuing the growth from L1 to adult stage requires 10-fold less mevalonate. GFP reporter strains revealed that protein prenylation and the distribution of muscle mitochondria are abnormal in both *hmgr-1* mutants as well as in nematodes treated with statins (Ranji *et al.* 2014).

Aside from HMG-CoA reductase, work in *C. elegans* has revealed that another regulated enzyme in the mevalonate pathway is HMG-CoA synthase. A recent study revealed that HMG-CoA synthase is regulated by post-translational ubiquination and age-related sumoylation (Sapir *et al.* 2014). The sumoylation levels increase with age, and sumoylation is balanced by the age-regulated activity of ULP-4, a SUMO protease (Sapir *et al.* 2014).

An interesting indirect role for the mevalonate pathway is its contribution to the silencing of genes by microRNAs (miRNAs). Reduction of activity of several genes, including *hmgr-1*, and *hmgs-1* in the mevalonate pathway by RNAi leads to inappropriate expression of several genes that are normally repressed by miRNAs (Shi and Ruvkun 2012). Specifically, the dolicol branch of the mevalonate pathway is responsible for proper silencing function of miRNAs. Dolicol is a lipid carrier of the oligosaccharide used for N-glycosylation of proteins, a process that is required for proper function of the miRISC complex (Shi and Ruvkun 2012).

The *C. elegans gro-1* gene encodes an isopentenylpyrophosphate:tRNA transferase (IPT), which modifies tRNAs (Lemieux *et al.* 2001). This modification is thought to increase the efficiency of protein translation (Persson *et al.* 1994). In *C. elegans*, GRO-1 is required maternally for normal rates of embryogenesis, postembryonic development, and normal brood size. Intriguingly, mitochondrially localized GRO-1 expression, but not cytoplasmic expression, rescues the developmental phenotypes of *gro-1* mutants (Lemieux *et al.* 2001).

Roles of coenzyme Q in C. elegans

Coenzyme Q is an electron carrier of the mitochondrial electron transport chain. Its reduced form, ubiquinol, acts as an

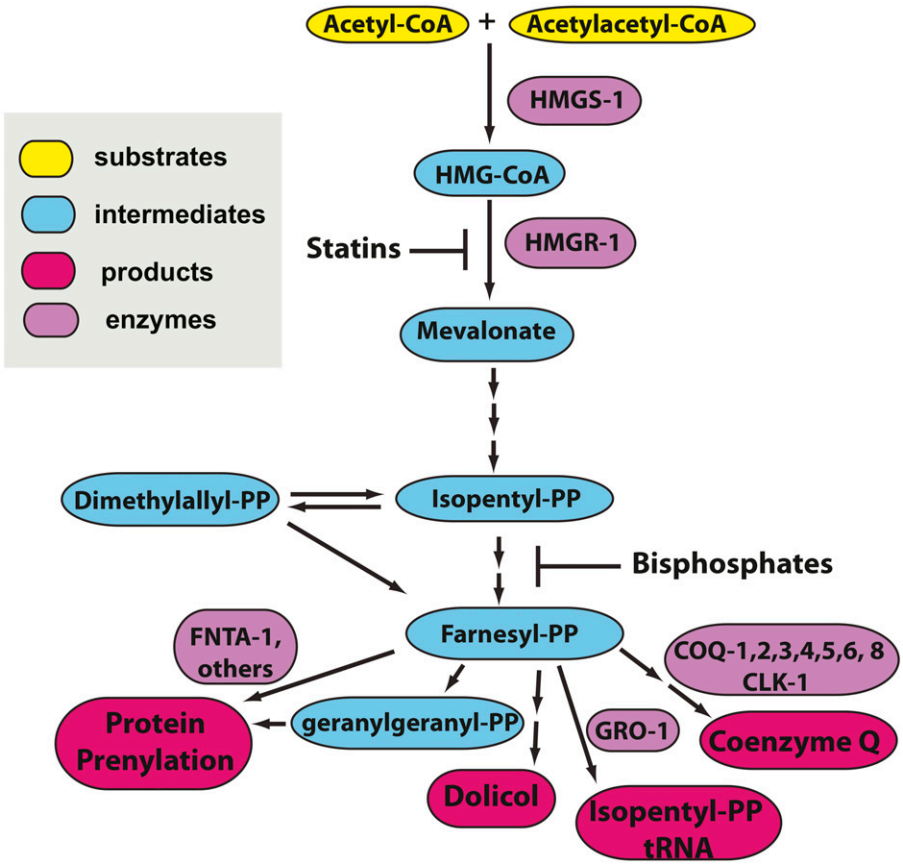

Figure 6 Abbreviated pathway of prenol and sterol lipid synthesis in *C. elegans*. Substrates are shown in yellow ovals, lipid intermediates are depicted in blue ovals, products are depicted in red ovals, and enzymes are shown in purple ovals. -PP, pyrophosphate.

antioxidant, inhibiting lipid peroxidation in most subcellular membranes, and recycling other antioxidants such as vitamin E and vitamin C (Ernster and Dallner 1995). The structure of coenzyme Q consists of a benzyoquinone ring that can be reversibly reduced and oxidized, with a prenylated tail of varying number of isoprene units. Bacteria predominately synthesize CoQ_8, while *C. elegans* and rodents synthesize CoQ_9, and humans predominately contain CoQ_{10}. Eight gene products are required for the synthesis of coenzyme Q from farnesyl PP. These are encoded by the *coq-1*-*coq-8* genes in *Saccharomyces cerevisiae* and *C. elegans*, although in *C. elegans* *coq-7* is known as *clk-1*, because mutants were isolated based on their slow development and long lifespan phenotypes (Wong *et al.* 1995; Ewbank *et al.* 1997; Larsen and Clarke 2002). While most mutant strains lacking an enzyme required for coenzyme Q biosynthesis die as larvae, the *clk-1* strain is viable. Even though *clk-1* mutants fail to synthesize CoQ_9, and instead accumulate dimethoxy CoQ, the CoQ_8 they obtain from their bacterial diet permits development and fertility (Jonassen *et al.* 2002, 2003). A *coq-8* deletion strain synthesizes ~7% of wild type levels of CoQ9, and mutants show developmental defects, including a detachment of the cuticle from muscle cells and reduced fertility (Asencio *et al.* 2009). A reduction of CoQ9 and related metabolites occurs during dietary restriction in *C. elegans*, and this reduction may be due to reduced expression of the coenzymeQ biosynthesis genes, including *coq-1* and *coq-5* (Fischer *et al.* 2015). Thus, endogenous coenzyme Q synthesis modulates both development and aging in *C. elegans*.

Roles of cholesterol in C. elegans

In mammals, cholesterol is an important membrane component required to regulate membrane fluidity, and an important structural component of membrane microdomains. Compared to other animals, *C. elegans* accumulates very low levels of cholesterol in cellular membranes (Matyash *et al.* 2001; Merris *et al.* 2003). Even though *C. elegans* lacks the ability to synthesize cholesterol (Vinci *et al.* 2008), they can readily absorb sterols from the diet, and *C. elegans* are routinely grown in the laboratory in the presence of cholesterol. Cholesterol depletion experiments indicate that very small amounts of cholesterol are required for molting and larval growth, and achieving depletion is difficult due to small amounts of sterols contaminating agar and other media ingredients (Kurzchalia and Ward 2003). Using organic solvent-extracted agar, *C. elegans* grow normally and are fertile until the F1 generation, which become arrested during larval growth, indicating that sterols are essential for *C. elegans* development.

Consistent with the requirement for small amounts of steroid precursors, bile-acid like steroid hormones and receptors are required for reproduction and dauer formation in *C. elegans*. Sterol-derived ligands were shown to bind the NHR DAF-12 (Motola *et al.* 2006). Synthesis of the ligands, called

Δ7 dafachronic acid and Δ4 dafachronic acid, whose structures resemble mammalian bile acids, require the activity of the DAF-9 CYP450, which hydroxylates cholesterol twice at the C26 position to form a carboxyl group (Gerisch *et al.* 2001; Jia *et al.* 2002). Other modifications require the DAF-36 Rieske-like monooxygenase, which acts as a 7-desaturase, to convert cholesterol to 7-dehydrocholesterol (Rottiers *et al.* 2006), as well as the HSD-1 hydroxysteroid dehydrogenase for synthesis (Patel *et al.* 2008). These and other steroid-derived ligands (Mahanti *et al.* 2014), together with the DAF-12 receptor, play a major role in regulating developmental and reproductive responses to the environment (Antebi 2015). Synthesis of bile acid ligands are regulated by NHR-8, a homolog of sterol-sensing receptors which acts upstream of DAF-12. NHR-8 regulates the transcription of genes involved in synthesizing dafachronic acids, fatty acid desaturation, and apolipoprotein production (Magner *et al.* 2013). During dietary restriction, which leads to longer lifespan in *C. elegans* and many other animals, levels of Δ7 dafachronic acid are increased, and signaling through NHR-8 and LET-363/mTOR mediates the lifespan extension (Thondamal *et al.* 2014). Thus, steroid hormone signaling is essential for development as well as lifespan regulation in *C. elegans*.

Introduction to Carbohydrates

Carbohydrates are hydrated organic molecules consisting of carbon (C), hydrogen (H), and oxygen (O), characterized by the formula $C_x(H_2O)_y$. Chemically, most carbohydrates are polyhydroxy aldehydes, ketones, alcohols, and acids. They are typically classified based on their structural complexity; the simplest moiety is a monosaccharide, a single molecule following the formula $C_x(H_2O)_y$ that cannot be hydrolyzed into smaller carbohydrates. Monosaccharides form polymers referred to as disaccharides, oligosaccharides, and polysaccharides, which consist of repeats monosaccharide moieties with acetal type linkages. These polymers follow the formula $[C_x(H_2O)_y]_n$, where $n = 2$ for disaccharides, $n = 2$–11 for oligosaccharides, and $n > 10$ for polysaccharides, which can reach up to several thousands of monosaccharide units. Many mono and disaccharides are known as sugars, due to their sweet taste.

Carbohydrates exist in all living kingdoms and have a variety of essential functions. Carbohydrates are a major energy source for all nonphotosynthetic organisms. Animals store glycogen, a polymer of thousands of monosaccharide units, while plants store long polymers of amylose and amylopectin. Other polysaccharides serve vital structural functions, such as chitin in animals, including *C. elegans*, and cellulose and hemicellulose in plants. Five-carbon carbohydrates such as ribose and deoxyribose are essential components of RNA and DNA, respectively. Ribose is essential for the biosynthesis of biochemical cofactors such as nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), adenosine mono/di/triphosphate (AMP/ADP/ATP), and others.

Functions and Metabolism of Monosaccharides

Monosaccharides are simple carbohydrates that are classified by the intramolecular position of the carbonyl (carbon–oxygen double bond) group, as well as the number and chirality of the carbons. Stereochemical chirality is defined by the position of the various hydroxyl-groups. Carbonyl groups can either be ketones, defining the corresponding sugar as a ketose, or aldehydes giving rise to aldoses. Thus, monosaccharides carry defining names such as aldohexoses, which include glucose.

Glucose transport, metabolism and function

D-Glucose (Figure 7A) is the best biochemically characterized monosaccharide, while its rare enantiomer L-glucose (Figure 7B) is inaccessible to enzymatic breakdown. The process of glycolysis breaks down D-glucose and converts it into lactate or pyruvate, to generate readily accessible energy equivalents including ATP (Figure 8). Besides β-oxidation and subsequent oxidative phosphorylation (OXPHOS), glycolysis is the most relevant energy-providing process in the animal kingdom, and it is highly conserved and well-characterized in *C. elegans* (Gebauer *et al.* 2016; Yilmaz and Walhout 2016). Unlike β-oxidation and OXPHOS, glycolysis does not necessarily require oxygen, and promotes nematodal survival in hypoxic states (LaMacchia *et al.* 2015).

Cellular glucose uptake in mammals is mediated by specific glucose transporters (GLUTs) (Herman and Kahn 2006). Recently, eight putative GLUT orthologs have been identified in *C. elegans*. These were characterized by heterologous overexpression in *Xenopus* oocytes (Feng *et al.* 2013; Kitaoka *et al.* 2013). Only one candidate gene, namely *fgt-1*, was found to mediate glucose uptake in both the oocyte expression system as well as in nematodes (Feng *et al.* 2013; Kitaoka *et al.* 2013). The *C. elegans* transporter appears to be less substrate-specific than the corresponding, and glucose-specific, GLUTs in mammals, since FGT-1 is capable of transporting other hexoses like fructose, galactose, and mannose (Kitaoka *et al.* 2013).

Following cellular uptake, glucose is either stored as glycogen, or metabolized by the glycolytic pathway (Figure 8). The early RNAi-based screening approaches have found that inhibition of the glycolytic enzyme glucose phosphate isomerase 1 (GPI-1) extends lifespan (Lee *et al.* 2003; Hamilton *et al.* 2005; Schulz *et al.* 2007), suggesting that reduced glucose metabolism promotes longevity. Consistently, blockade of glycolysis with chemical agents like 2-deoxyglucose, glucosamine, or RNAi-mediated knock-down of *fgt-1* also extends lifespan of nematodes (Schulz *et al.* 2007; Feng *et al.* 2013; Weimer *et al.* 2014) and mice (Weimer *et al.* 2014), respectively.

In mammals, a significant portion of glucose uptake depends on insulin signaling-mediated and translocation-dependent activation of GLUT4 (Huang and Czech 2007). In *C. elegans*, impaired insulin/IGF-1/INS-18 signaling extends lifespan (Friedman and Johnson 1988; Kenyon *et al.* 1993), and, consistent with mammalian insulin signaling, reduces glucose uptake (Zarse *et al.* 2012). Conversely, impairing nematodal glucose

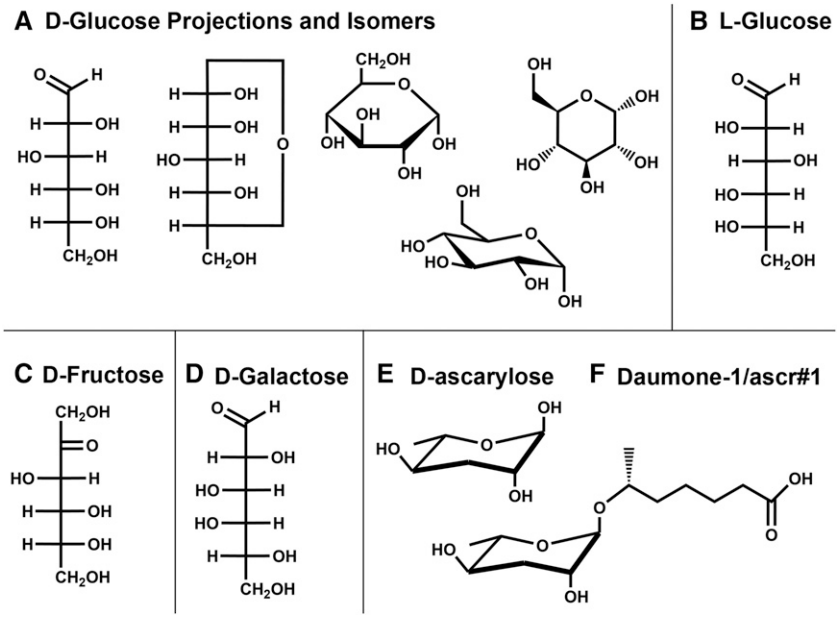

Figure 7 Structures of glucose and other monosaccharides. (A) Various projections and isomers of D-glucose. Fisher projections of the respective open-chain forms of (B) L-glucose, (C) D-fructose, and (D) D-galactose, respectively. Structures of (E) the dideoxy sugar ascarolose and (F) the ascaroside daumone-1, also known as ascr#1.

transport by inhibition of FGT-1 epistatically interacts with AGE-1 and DAF-2 signaling regarding extension of lifespan, indicating that impaired glucose uptake by knock-down of the transporter (*fgt-1* RNAi) and impaired insulin/IGF-1 (AGE-1, DAF-2) likewise reducing glucose share a functional denominator (Feng *et al.* 2013).

Early approaches to establish axenic media conditions demonstrated than *C. elegans* can be maintained on glucose as the only energy source, as well as on trehalose (a glucose dimer, see below) and glycogen (a glucose polymer, see below) (Lu and Goetsch 1993). By contrast, other saccharides like fructose or sucrose exerted toxicity. Also, high concentrations of trehalose and glucose inhibited growth (Lu and Goetsch 1993). Researchers have repeatedly demonstrated that high, *i.e.*, millimolar, concentrations of glucose (in addition to the normal *E. coli* OP50 food source) impair the lifespan of nematodes (Schulz *et al.* 2007; Lee *et al.* 2009, 2015; Schlotterer *et al.* 2009; Choi 2011; Fitzenberger *et al.* 2013; Deusing *et al.* 2015a,b; Dobson *et al.* 2017). Notably, this effect is not observed in males (Miersch and Doring 2012; Liggett *et al.* 2015), possibly due to sex-specific differences in expression of genes involved into carbohydrate catabolism (Miersch and Doring 2012).

Experimental evidence points to several mechanisms mediating the lifespan reduction by glucose. Reducing glycolysis has been shown to induce mitochondrial OXPHOS to generate a lifespan-extending reactive oxygen species (ROS) signal (Schulz *et al.* 2007) due to a process called mitohormesis (Ristow 2014) that has been confirmed in multiple organisms, including *C. elegans* (Zarse *et al.* 2012; K. Schmeisser *et al.* 2013; S. Schmeisser *et al.* 2013; De Haes *et al.* 2014; Tauffenberger *et al.* 2016). Conversely, increased glucose levels reduce both OXPHOS and ROS, impairing lifespan (Schulz *et al.* 2007; Zarse *et al.* 2012). Independently, it has been shown that increased glucose concentrations direct excess glucose-6-phosphate into the hexosamine pathway causing formation of methylglyoxal and advanced glycated end products to limit longevity (Morcos *et al.* 2008; Schlotterer *et al.* 2009; Chaudhuri *et al.* 2016). Third, because glucose affects formation of the metabolic intermediate glycerol, the aquaporin AQP-1 has been linked to osmotic stress tolerance and lifespan regulation following exposure to glucose (Lee *et al.* 2009). Fourthly, and since blocking GPI-1 by RNAi or chemical inhibitors forces glucose-6-phosphate into the pentose phosphate shunt (Figure 8), subsequent alterations in nematodal redox state have been proposed to mediate the phenotype (Ralser *et al.* 2008; Kruger *et al.* 2011; Penkov *et al.* 2015), given that the pentose phosphate shunt is responsible for generation of NADPH, which, in turn, affects levels of reduced glutathione (GSH), besides others. Along this line, it was subsequently shown that depletion of NADPH also initiates dauer formation due to impaired biosynthesis of dafachronic acid from cholesterol—a NADPH-dependent process (Penkov *et al.* 2015). Consistently, it was shown that carnitine protects from glucose toxicity in a DAF-12-dependent manner, notably the receptor for dafachronic acid (Deusing *et al.* 2015a). Other mechanism that ameliorate glucose toxicity are (i) modulation of membrane lipid composition via PAQR-2 and IGLR-2 (Svensk *et al.* 2016), (ii) reduction of glucose-mediated triglyceride accumulation via SREBP and MDT-15 (Lee *et al.* 2015), and lastly O-linked N-acetylglucosamine cycling (Love *et al.* 2010; Mondoux *et al.* 2011). Lastly, exposure to glucose reduces fecundity in a transgenerational manner (Tauffenberger and Parker 2014).

Despite this extensive body of evidence regarding glucose toxicity and its underlying mechanisms, excess glucose has unexpectedly been demonstrated to reduce proteotoxicity in several *C. elegans* models of protein aggregation (Tauffenberger *et al.* 2012). It remains to be shown how this interesting observation

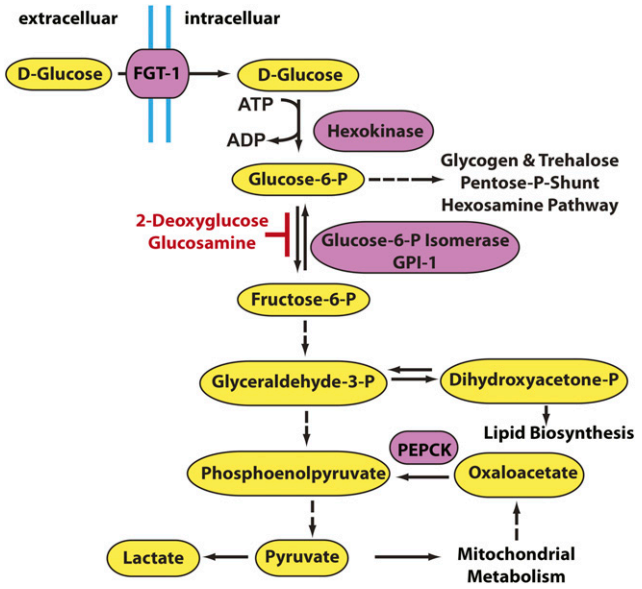

Figure 8 Glucose uptake, glycolysis and interacting pathways. In *C. elegans*, glucose is taken up by the FGT-1 glucose transporter. Glucose is phosphorylated by hexokinase and enters various metabolic pathways, including glycolysis, glycogen synthesis, and interacting pathways. –P, phosphate; PEPCK, phosphoenolpyruvate carboxykinase.

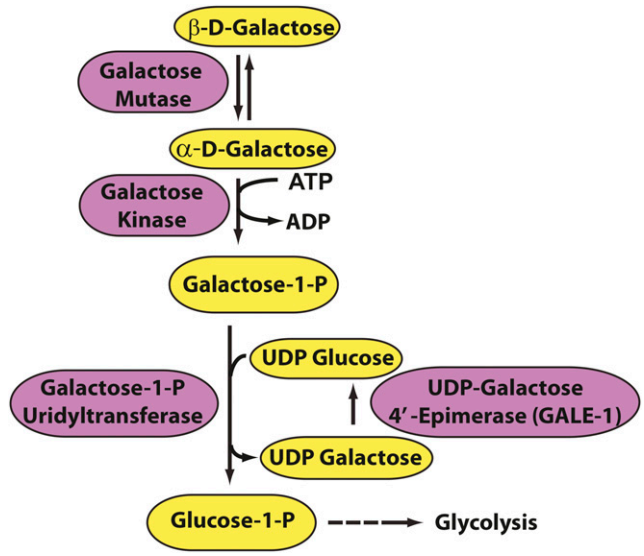

Figure 9 Galactose metabolism. The conserved Leloir-pathway converts galactose to glucose-6 phosphate using the activity of four key enzymes.

translates into higher organisms, particularly since diabetes mellitus, and hence elevated glucose levels, have been linked to increased incidence of Alzheimer's disease in multiple human cohorts (Zhang *et al.* 2017).

Fructose and galactose

As stated above, in comparison to glucose and glucose-derived di- and polymers, the monosaccharide fructose (Figure 7C) has been shown to be significantly less effective in maintaining viability of nematodes in axenic culture (Lu and Goetsch 1993). Very recently, fructose has been shown to extend lifespan at lower doses while higher doses shorten lifespan, both in liquid culture and due to unknown mechanisms (Zheng *et al.* 2017). Moreover, fructose at lower doses appears to reduce intestinal lipid deposition, while higher doses induce the opposite, potentially reflecting the effects of this sugar on hepatic lipid accumulation in mammals (Zheng *et al.* 2017).

Galactose (Figure 7D) is a monosaccharide that serves as an important component in the formation of—widely undefined—glycolconjugates, *i.e.*, the plethora of lipid or protein molecules with at least one sugar moiety attached to it. While beyond the primary/metabolic scope of this text, such changes will be discussed below.

Independently, galactose metabolism has been analyzed in *C. elegans*, and essentially reflects the so-called Leloir pathway (Holden *et al.* 2003), where galactose is converted into glucose derivatives to make it accessible for glycolytic catabolism (Figure 9). Enzymatic defects of this pathway have been linked to an inherited human disease named galactosemia. The Leloir pathway requires four enzymes: the first step is the conversion of β-galactose into to α-galactose by an enzyme called galactose mutarotase, which has been crystallized for both humans and *C. elegans* (Thoden *et al.* 2004). The second step is catalyzed by galactokinase to yield galactose-1-phosphate. The third enzyme is galactose-1-phosphate uridyl-transferase, which transfers an UMP group from UDP-glucose to galactose-1-phosphate, thereby generating glucose 1-phosphate and UDP-galactose. The last step is the conversion of UDP-galactose into UDP-glucose by UDP-galactose 4-epimerase, named GALE-1 in nematodes. Complete loss-of-function mutations of *gale-1* cause lethality, while partial loss-of-function mutants are viable (Brokate-Llanos *et al.* 2014). Moreover, the authors observe hypersensitivity to galactose supplementation, as well as to infections in partial loss-of-function mutants of *gale-1*, as well as interactions with the unfolded protein response (Brokate-Llanos *et al.* 2014).

Sugar alcohols

Sorbitol (Figure 10A) is an alcohol produced from glucose by the enzyme aldose reductase, which, in mammals, occurs as a consequence of supraphysiologically increased glucose levels. This reaction requires NADPH, and may hence impair redox defense of the cell by depleting GSH (see above). Sorbitol can further be metabolized to fructose by sorbitol dehydrogenase, which requires NAD+ as a cofactor. Collectively, these two enzymatic steps are known as the polyol pathway (Figure 10B). Sorbitol was found to be most strikingly elevated in a global screen for aging-associated metabolites in *C. elegans*, and, consistently, a change of redox state was observed in the same experimental setting (Copes *et al.* 2015).

By contrast, sorbitol supplementation has been shown to extend nematodal lifespan, likely due to induction of osmotic stress and subsequent induction of stress response pathways (Chandler-Brown *et al.* 2015; Hipkiss 2015). The same

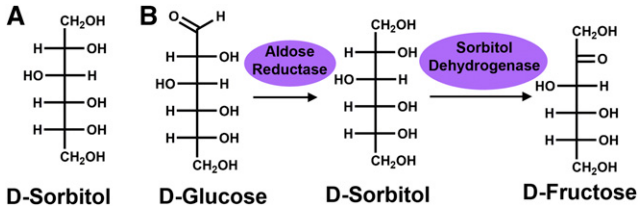

Figure 10 Sorbitol and the polyol pathway. (A) The structure of the sugar alcohol sorbitol. (B) The interconversion of glucose, sorbitol, and fructose by the polyol pathway.

mechanism may also apply to fructose-mediated lifespan extension (see above) (Zheng *et al.* 2017), as well as to the concomitant effects of the disaccharide trehalose (see below).

Dideoxysugars (ascarosides)

Ascarosides are glycolipids that, by definition, always contain the dideoxysugar ascarylose (3,6-dideoxy-L-arabino-hexose) (Figure 7E) linked to a fatty acid of varying chain length. One well-characterized example is the first ascaroside isolated from *C. elegans*, daunome-1, also known as ascr#1, which carries a saturated C7 fatty acid, *i.e.*, enanthic or heptanoic acid (Figure 7F). Fatty acids linked to ascarylose can be saturated (as in daunome-1), or unsaturated. Ascarosides were initially identified in the roundworm family *Ascaridia spp*., and have been subsequently identified in *C. elegans* to function as extracellular signaling molecules. In the latter, ascarosides control the dauer state as well as male attraction, hermaphrodite repulsion, olfactory plasticity, and aggregation. They act mainly through G protein-coupled receptors (GPCRs) (Pungaliya *et al.* 2009; Ludewig *et al.* 2013), and have been excellently described elsewhere in this publication (Ludewig and Schroeder 2013).

Functions and Metabolism of Disaccharides, Oligosaccharides, and Polysaccharides

Trehalose

Disaccharides, by definition, contain two monosaccharides formed by glycosidic linkage, *i.e.*, by a condensation reaction at two hydroxyl groups. While sucrose, lactose, and maltose are the most relevant disaccharides in mammalian biology, trehalose (Figure 11A) is the only disaccharide that has been characterized in nematodes to a significant extent.

Trehalose is found in many lower organisms, including plants, fungi, and metazoae, and serves different purposes importantly including energy storage and stress resistance. Chemically, trehalose is formed from two molecules of D-glucose by an α,α-1,1-glucoside bond (Figure 11B). Its precursor, trehalose-6-phosphate, is synthesized from glucose-6-phosphate and UDP-glucose by an enzyme called trehalose-6-phosphate synthase, encoded by *tps-1* and *tps-2* in *C. elegans*. Impairing expression of both *tps* genes reduces trehalose content in *C. elegans* by >90% (Pellerone *et al.* 2003). Trehalose-6-phosphate is next dephosphorylated by an enzyme named trehalose-6-phosphate

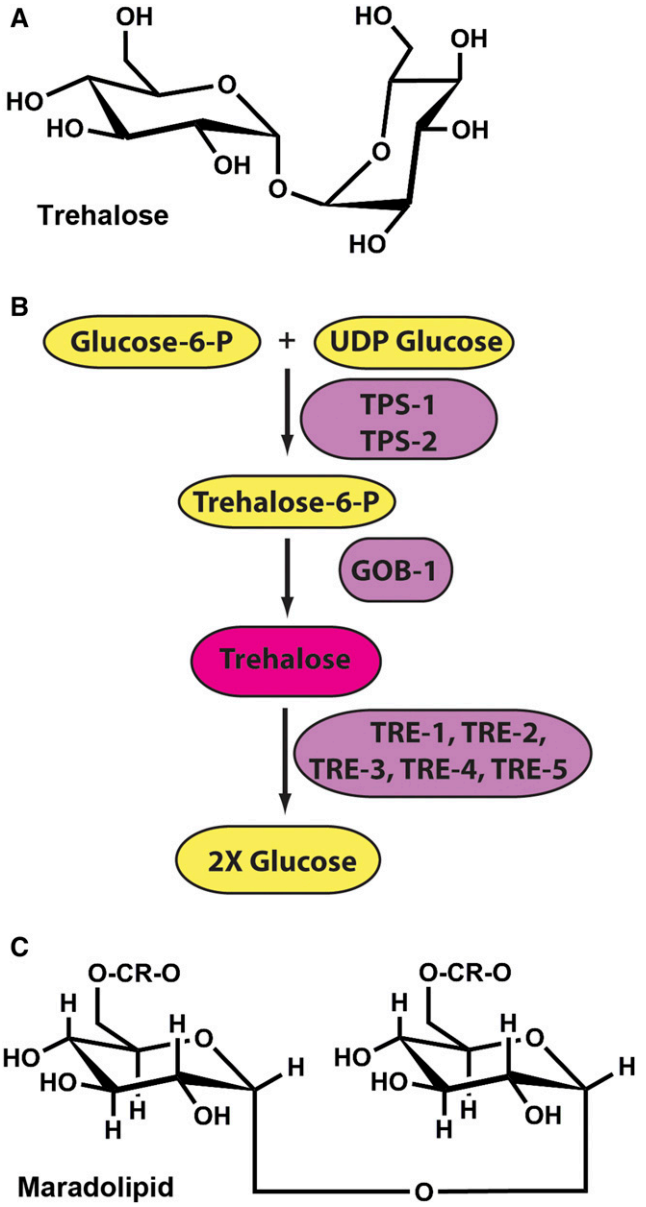

Figure 11 Structure and metabolism of trehalose and maradolipids. (A) Structure of the disaccharide trehalose. (B) Biosynthesis and catabolism of trehalose. Trehalose-P synthase is encoded by *tps-1* and *tps-2* genes, Trehalose-6-phosphate phosphatase is encoded by *gob-1*, and Trehalase is encoded by *tre-1-5* genes (C) General structure of maradolipids. R, fatty acyl chains, which frequently are branched (see text for details).

phosphatase (TPP), which, in *C. elegans*, is encoded by *gob-1*. While loss-of-function mutants of *gob-1* are lethal, impairment of *tps-1* and *tps-2* reverts *gob-1* lethality (Kormish and McGhee 2005). This indicates that accumulation of trehalose-6-phosphate, rather than lack of trehalose, causes lethality in nematodes.

Like glycogen, nematodes store trehalose to conserve energy (Roberts and Fairbairn 1965), and the disaccharide is made reavailable to glycolysis by cleavage to glucose mediated by an enzyme called trehalose (Figure 11B). This enzyme has been proposed to be encoded by the *tre* gene family

in *C. elegans* (Pellerone *et al.* 2003). Consistent with its role in energy storage, trehalose has been involved in the hatching process in nematodes (Perry and Clarke 1981). Nevertheless, the trehalose-to-glucose ratio is 5.4 times higher in males than in hermaphrodites (Miersch and Doring 2012).

Trehalose has a long-standing role as a stress protectant in various species (Van Laere 1989). Consistently, trehalose feeding extends *C. elegans* lifespan, and *daf-2*-mediated extension of lifespan is reduced by impairing *tps-1* or *tps-2* (Honda *et al.* 2010), while *daf-2* mutants show increased levels of *tps-1 and -2* mRNA expression (Lamitina and Strange 2005; McElwee *et al.* 2006; Honda *et al.* 2010; Zarse *et al.* 2012). Moreover, *C. elegans* increases endogenous trehalose production in states of extreme desiccation to ensure dauer survival in states of anhydrobiosis (Erkut *et al.* 2011). Preconditioning by mild desiccation for 4 days potentiated survival rates in states of extreme desiccation. Interestingly, mutants unable to synthesize trehalose lost the ability to survive desiccation in the dauer state, indicating that trehalose is not only associated with, but also the cause of, survival in states of anhydrobiosis (Erkut *et al.* 2011). Subsequently, the same authors showed that trehalose biosynthesis in states of desiccation mainly depends on the glyoxylate shunt, where sugars are synthesized from fatty acids, particularly acetate (Erkut *et al.* 2016), a mechanism also observed in *S. cerevisiae*, with possible translation into mammals in states of ketosis (Hu *et al.* 2014). The NADPH-dependent biosynthesis of trehalose during adverse conditions may also initiate dauer formation, since it was shown that depletion of NADPH promotes dauer formation due to impaired biosynthesis of dafachronic acid from cholesterol, a NADPH-dependent process (Penkov *et al.* 2015).

While trehalose is absent in mammals, trehalose supplementation nevertheless induces autophagy and prevents hepatic steatosis in murine cells, possibly by inhibiting the SLC2A protein (DeBosch *et al.* 2016). Moreover, trehalose has been successfully used for cryopreservation of mammalian and particularly human cells (Eroglu *et al.* 2000; Guo *et al.* 2000).

Maradolipids

Fatty acids can form condensation products with sugars—a process contributing to the glycosylation of membranes and other moieties. The disaccharides trehalose can form specific condensation products with two—typically different—types of fatty acid molecules. These condensation products have been named maradolipids (Penkov *et al.* 2010) and chemically reflect 6,6′-di-O-acyl-trehaloses (Figure 11C). Typically, one of the acyl moieties reflects oleic acid (18:1n-9), while the other moiety appears to be a branched-chain fatty acid of variable molecular structure (Penkov *et al.* 2010). Maradolipid-like components are widely found in plants and prokaryotes, and contribute to desiccation resistance in these species (Harland *et al.* 2008). Consistently, maradolipids are preferentially synthesized during dauer formation in *C. elegans* (Penkov *et al.* 2010). Consistent with the fact that maradolipid synthesis requires branched-chain fatty acids, RNA interference against *elo-5*, which is required for formation of such fatty acids (Kniazeva *et al.* 2004, 2008), prevents maradolipid biosynthesis in nematodes (Penkov *et al.* 2010). Likewise, impairing enzymes like *tps-1* or *tps-2* that are required for trehalose biosynthesis (Honda *et al.* 2010) impairs formation of maradolipids (Penkov *et al.* 2010).

Glycogen

In addition to trehalose, glycogen is the main carbohydrate storage in *C. elegans*. Glycogen consists of up to several thousands of glucose molecules, which can be released from glycogen by an enzymatic process called glycogenolysis. Inversely, excess glucose is stored as glycogen by activation of glycogen synthase (Figure 8). There is longstanding evidence for glycogen storage as well as glucose mobilization from glycogen in *C. elegans* (O'Riordan and Burnell 1989). In the dauer state, nematodes not only accumulate significant amounts of lipids (see above) but also glycogen (Popham and Webster 1979). Consistent with its role as energy buffer, glycogen is preferentially mobilized in the dauer state, while glycogen synthesis is downregulated (O'Riordan and Burnell 1989; Holt and Riddle 2003; Depuydt *et al.* 2014). Since glucose can be used to generate ATP in the absence of oxygen by means of anaerobic glycolysis (O'Riordan and Burnell 1989; Braeckman *et al.* 2009) (Figure 8), it has been consistently demonstrated that increased glycogenolysis mediates survival in states of anoxia (Frazier and Roth 2009; Larue and Padilla 2011; Depuydt *et al.* 2014; LaMacchia *et al.* 2015) and hypo-osmotic stress (LaMacchia *et al.* 2015).

Chitin

Chitin is a long-chain polymer of N-acetyl-glucosamine. The latter chemically qualifies as an N-glycosylated alanine (see below). N-Acetyl-glucosamine is derived from glucose through the hexosamine pathway, and has been shown to extend lifespan in *C. elegans* by inducing protein quality control (Denzel *et al.* 2014).

Chitin is an important structural component of *C. elegans*. Chitin is polymerized from N-acetyl-glucosamine monomers by two isoforms of chitin synthase, *chs-1* and *chs-2* (Veronico *et al.* 2001): *chs-1* mediates chitin deposition into the eggshell, and is expressed during later larval stages and adulthood, while *chs-2* provides chitin for the nematodal pharynx, and is expressed before the molt (Veronico *et al.* 2001; Zhang *et al.* 2005).

Glycosylation

Glycosylation is defined as the covalent addition of a carbohydrate molecule to lipid, nucleic acids, or protein moieties, leading to characteristic and functionally relevant modifications. Often oligosaccharides, consisting of >2 but <11 monosaccharide units, contribute to the glycation of proteins and lipids. Glycosylation is a specific and enzyme-directed process, and genetic defects in glycosylation cause numerous inherited diseases in humans. Particularly, the addition of sugars to the N- or the C-terminus of proteins to form N- and O-glycans has been intensely studied. Glycosylation of lipids typically reflects O-linked glycosylation. The numerous specific aspects of glycosylation in *C. elegans* have been excellently reviewed elsewhere (Berninsone 2006).

Summary and Future Directions

Metabolic pathways of lipid and carbohydrate metabolism are well conserved across the animal kingdom, including *C. elegans*. Interestingly, *C. elegans* also possess pathways that are rare in animals, but common in plants, such as the ability to synthesize polyunsaturated fatty acids in the absence of essential dietary fatty acids, and the synthesis of maradolipids for protection from desiccation. Specific lipids and carbohydrates play vital roles in development, and *C. elegans* research has been especially useful for unraveling the roles of lipids and carbohydrates during aging and stress. As in other animals, lipid and carbohydrate metabolism pathways converge, because animals have a limited capacity to store dietary carbohydrates, and thus carbons from dietary carbohydrates are converted to acetyl-CoA, where they can be diverted into fatty acid synthesis. The consequences of disrupting this linkage are devastating, as glucose toxicity is greatly elevated in both worms and humans when fatty acid synthesis regulators and pathways are disrupted (Lee *et al.* 2015; Listenberger *et al.* 2003).

Future research in lipid and carbohydrate metabolism in *C. elegans* will benefit from the rapid improvement of metabolomic analysis. In addition to lipids and carbohydrates, it will be important to analyze the metabolism of amino acids and intermediates of other metabolic pathways with respect to various developmental and environmental manipulations. Because the *C. elegans* diet consists of bacteria, and the major macronutrient by weight in bacteria is protein, it will be useful for future research to focus on catabolic and anabolic pathways of amino acid metabolism, including the interplay between amino acid breakdown products and lipid and carbohydrate metabolism. Combined with the powerful genetic approaches common in *C. elegans* research, this will lead to more insights into mechanisms of energy homeostasis and roles for macronutrients in development, aging, and stress responses.

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