Chapter 14
Lipid Metabolism, Lipid Signalling and Longevity

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Abstract Ageing research gains more attention as the aged population increases worldwide and ageing-related diseases become more prevalent. Model organism research in the last three decades has shown that ageing is regulated via several genetic pathways and environmental interventions, most of which are evolutionarily conserved. *C. elegans* has been the powerhouse of ageing research since the discovery of mutant strains with doubled lifespan. Interestingly, the pathways that regulate *C. elegans* ageing often affect lipid biology as well. This chapter will focus on the interaction between lipid biology and ageing by introducing well-known pathways that regulate ageing and how lipid levels, composition or distribution change when these pathways are defective. Last but not least, the signalling role for lipids in ageing will be discussed.

Keywords Lipid molecules • Lipid signalling • Longevity

14.1 Introduction

Ageing is an inevitable part of life, and until recently, it was thought to be a passive phenomenon that leads to decrease in organismal functions and fitness. However, elaborate research in the last three decades has shown that ageing is a complex process that is regulated by both intrinsic signalling pathways and extrinsic

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environmental stimuli [1]. Studies using model organisms such as yeast, nematodes, fruit flies and mice discovered that several genetic pathways and environmental interventions, which regulate ageing, are evolutionarily conserved and hold great promise for human ageing research.

Since the discovery that mutations in the *C. elegans* insulin receptor, DAF-2, can lead to doubling of lifespan, worms have become the prominent force in ageing research [1]. In addition to their short lifespan, *C. elegans* are transparent which renders them beneficial for staining techniques and microscopy imaging. Last but not least, the vast number of tools available for genetic manipulation as well as the ease of performing high-throughput genetic screens has enabled researchers to find several key players of signalling pathways regulating longevity and their detailed epistasis analysis.

Interestingly, many of the pathways that regulate longevity affect lipid biology as well. For example, *C. elegans* mutants that lack normal DAF-2 activity also have increased lipid levels [2, 3]. However, there is no simple correlation between pro-longevity pathways and increased lipid storage levels. *eat-2* mutants, genetic models of dietary restriction in *C. elegans*, are long-lived, but have decreased lipid storage [4]. Thus, the involvement of lipids in ageing is more complicated than expected. Apart from their role in energy storage, lipids are also important signalling molecules. Examples include, ceramides and certain fatty acids, which have been shown to be important for ageing [5, 6]. More studies on the characterization of the role of lipid biology in longevity will advance our knowledge in the biology of ageing and improve strategies for therapeutic interventions for healthy ageing.

In this chapter, we will focus on the general concepts of lipid biology and lifespan-regulating pathways with an emphasis on *C. elegans* longevity mutants and their lipid metabolism. We will also provide an overview of lipid analysis methodologies. Finally, we will mention more recent research on the role of lipids as signalling molecules.

14.2 Lipids

Lipids are a diverse class of small, organic molecules that are either amphipathic or hydrophobic [7]. Lipids are perhaps most associated with their roles as a source of energy storage and accumulation during obesity. Other than storing energy, lipids play major roles in forming membranes to mark the boundaries of a cell and to separate cellular compartments. Besides their well-known structural functions, lipids are also biologically active molecules providing communication within and between cells [8]. These signalling roles have implications in several diseases, such as various types of cancer and metabolic syndromes [9], as well as regulating healthy ageing [10].

14.2.1 Structure and Classification

Because lipids are such a broad grouping of molecules, they have been classified into categories, each of which has multiple subclasses [7]. These categories are: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides [7]. Within these groups, different lipids are distinguished from each other by a variety of criteria, including their length, the number and location of double bonds in their hydrocarbon tail(s), and the attached structure(s), such as phosphates or glycerol. Four groups of lipids are focused in this chapter to demonstrate the diversity between different lipid categories (Fig. 14.1).

14.2.1.1 Fatty Acyls

Fatty acyls are a diverse group of lipids that include the major sub-grouping of fatty acids, which are carboxylic acids with a hydrocarbon tail [11]. Based upon the number of double bonds in the hydrocarbon tail, fatty acids can be further divided into saturated (no double bonds), monounsaturated (a single carbon-carbon double bond), and polyunsaturated fatty acids (more than one carbon-carbon double bond).

The saturated fatty acids usually contain 14–22 carbon atoms. The monounsaturated fatty acids are also similar length, but they have a carbon-carbon double bond, commonly in *cis*-configuration, which means the hydrogen bonds next to the double bond are positioned in the same direction. The presence of the double bond gives the molecule a "kink" in its shape, which changes its biochemical properties. Polyunsaturated fatty acids (PUFAs) contain several carbon-carbon double bonds, and they are named depending on the location of the first double bond: they are called ω-3 fatty acids if the bond is between the third and the fourth carbon after the ω-carbon and ω-6 if the bond is between the sixth and seventh. Two PUFAs, linoleic acid and alpha-linolenic acid are essential nutrients for mammals, but unlike mammals, *C. elegans* express the desaturases (*fat-1* and *fat-2*) that are necessary to synthesize these PUFAs *de novo* [12].

Fatty acids are important energy fuels for the cell, which can be degraded via β–oxidation to generate acetyl-CoA and subsequently used to generate ATP via the citric acid cycle [13]. Fatty acids and their derivatives are crucial for cellular homeostasis and organism fitness, and they can be utilized in both intracellular and extracellular signalling. Research in the last decade showed that dietary ω-3 fatty acids are involved in both neurotransmission and neurogenesis, and may also be important for preventing age-related brain damage and neurodegenerative diseases, such as Alzheimer's disease [14]. Even though the complete mechanism is unclear, these studies showed that ω-3 fatty acids regulate microglia and astrocyte activity, improve mitochondrial functions, and reduce oxidative damage [14]. More recently, a specific monounsaturated fatty acid derivative, oleoylethanolamide (OEA), was shown to be involved in longevity regulation at the organismal level. This *C. elegans* study showed that OEA acts as a signalling molecule between the lysosomes and the

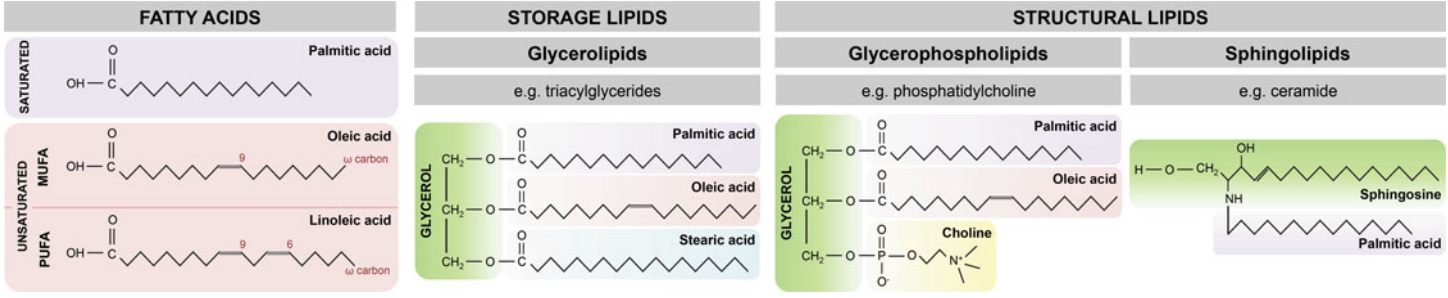

Fig. 14.1 Different classes of lipid species. Fatty acids, which are carboxylic acids with a hydrocarbon tail, are building blocks of many other lipid species. Depending on the number of double bonds in the hydrocarbon tail, they are classified into three groups: saturated, mono-unsaturated (*MUFA*) and poly-unsaturated (*PUFA*). Glycerolipids consist of a glycerol backbone attached to one-to-three fatty acids. For example, triacylglycerols have glycerol attached to three fatty acids, which can have hydrocarbon tails of different sizes and saturation. Triacylglycerols are the major source of energy storage in cells. Glycerophospholipids are lipids with a polar group attached to one of the positions on the glycerol backbone and fatty acids attached to the other two. For example, phosphatidylcholine has a choline attached to the third position on the glycerol. Sphingolipids are membrane-associated lipids with a sphingoid base attached to one fatty acid. For example, ceramides have sphingosine attached to a fatty acid

nucleus where it will activate specific nuclear hormone receptors to induce longevity [10]. OEA is part of a larger group of fatty acid derivatives known as N-acylethanolamines (NAEs). Another NAE, eicosapentaenoyl ethanolamide (EPEA), has been shown to be able to modulate organism lifespan via dietary-restriction [15]. Additionally, fatty acids have roles in extracellular signalling. Several free fatty acids (FFA) have been shown to regulate insulin secretion in mammalian cell lines via G-protein coupled receptor (GPCR) signalling [16]. More specifically, palmitoleate (C16;1n7) acts as a lipokine derived from the adipose tissue to improve insulin and glucose metabolic homeostasis in the muscle and liver systematically [17].

14.2.1.2 Glycerolipids

Glycerolipids are lipids with a glycerol backbone with one to three attached fatty acids [11]. There are mono-, di- or tri-acylglycerols depending on the number of attached fatty acids. Each of the fatty acids in diacylglycerols (DAGs) or in triacylglycerols (TAGs) can be different.

TAGs are the major intracellular source of energy storage, and they can be degraded by lipases in the presence of the proper signals, such as a demand for energy, resulting in the release of free fatty acids (FFAs). Homeostasis in lipid storage, especially the level of TAGs, is essential for healthy ageing since obesity is associated with age-related diseases such as cardiovascular disease, type II diabetes and certain types of cancer [18]. However, as mentioned in the introduction, there may not be a simple correlation between overall lipid levels and organism longevity. Worms under dietary restriction have lower lipid storage [19] whereas insulin-receptor deficient worms, *daf-2* mutants, have more [20], but yet both worm strains are long-lived. Therefore, future studies should investigate whether differential distribution of TAGs in certain tissues affect ageing. It is also possible that the composition and not the overall level of TAGs affect longevity.

DAGs, on the other hand, have a much more diverse set of roles. Many intracellular pathways use DAGs as a second messenger by binding to a group of proteins with a C1 domain such as protein kinase C [21] and indirectly regulate the activities of G proteins [22]. Therefore, DAGs have important roles in processes such as proliferation, apoptosis, differentiation, and cellular migration [23]. DAGs also affect the physical aspects of membranes, such as their structure and dynamics, as well as function in lipid metabolism by either being degraded to generate FFAs or added to other lipids in order to generate more complex lipids [21]. DAG metabolism is also involved in ageing. In flies and worms, knockdown of diacylglycerol lipase or overexpression of the diacylglycerol kinase extends lifespan [24]. The same study suggested that DAG metabolism interacts with TOR signalling to regulate longevity.

14.2.1.3 Glycerophospholipids

Glycerophospholipids are lipids that have a phosphate group connecting a polar group to a glycerol backbone, with fatty acids attached to the other two positions on the glycerol [11]. Similar to the glycerolipids, the fatty acids attached to the glycerol can be of any kind. But mostly they contain a saturated fatty acid in the first carbon and an unsaturated fatty acid in the second carbon. The third carbon is joined to a polar alcohol with a phosphodiester bond. Like all of the lipid molecules in the cell membrane, glycerophospholipids are also amphipathic.

Glycerophospholipids are the main component of the membrane bilayer. In addition to this commonly known role, they play an important role in signalling. One of the best-known phospholipid signalling molecules is the phosphotidylinositols. Phosphotidylinositols are a subclass of phospholipids that are partially located within the membrane. Certain cell-surface G-protein coupled receptors (GPCRs) activate phospholipase C (PLC), which then cleaves phosphotidylinositols to produce diacylglycerol (DAG) and inositol phosphates [25]. Both DAG and inositol phosphates have signalling capabilities and affect a plethora of aspects of the cell, including Ca^{2+} release, lipid transport and membrane dynamics [26].

Ageing increases the cholesterol/phospholipid ratio in cell membranes, thus decreasing the membrane fluidity. Lipofuscins, sometimes called age pigments, are derived from the peroxidation of subcellular membrane lipids containing PUFAs and precipitate in the lysosomes. Lipofuscin accumulation is also implicated in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Phosphoinositide signalling cascade is also affected at many levels during the ageing process, such as receptor availability and kinase activity [27].

14.2.1.4 Sphingolipids

Sphingolipids are membrane-associated lipids that have a sphingoid base. These are then built upon and modified to become more complex lipids, such as ceramides [11]. Ceramide is important in multiple aspects of programmed cell death (PCD) [28, 29]. It can affect intrinsic and extrinsic PCD-related signalling pathways as well as both caspase-dependent and caspase–independent mechanisms [30]. Ceramide also plays a crucial role in other developmental processes such as differentiation of the primitive ectoderm in embryos and asymmetric cell division [31]. As worms develop and age, sphingolipids naturally accumulate, and so inhibition of the synthesis and accumulation of sphingolipids leads to a delay in the development and ageing of *C. elegans* [32]. Interestingly, loss-of-function mutations in genes encoding the ceramide-synthesis enzymes, *lagr-1* and *sphk-1*, results in increased autophagy and extension in lifespan [33].

14.2.2 Synthesis, Storage, and Degradation of Lipids

Lipids can be either synthesized *de novo* or absorbed from the diet (Fig. 14.2). The starting point of *de novo* synthesis is acetyl CoA, which will be extended into malonyl-CoA by acetyl-CoA carboxylase and further into palmitic acid by fatty acid synthase [34]. Then, palmitic acid, a 16 carbon saturated fatty acid, can be further elongated by the enzymes ELO-1, ELO-2, and LET-767, and/or desaturated by the enzymes FAT-1 to FAT-7. Desaturation is possible in *C. elegans*, but possible only to a limited extent in mammals [12, 34]. These fatty acids then serve as the base for many lipids. For example, coenzyme A-bound fatty acids (acyl-CoA) can be combined with glycerol-3-phosphate, a phosphorylated glycerol, to generate lysophosphatidic acid (LPA). A second acyl-CoA can be added to LPA to generate phosphatidic acid, which can have its phosphate removed to generate DAG. A third acyl-CoA can be incorporated to then generate TAG [35].

While *de novo* synthesis is extremely important, not every organism can synthesize every lipid. This makes the dietary intake of lipids a key aspect in maintaining lipid homeostasis and general organismal functions. One notable example of the importance of dietary intake of lipids in *C. elegans* is cholesterol, which is a key component of membrane structures and signalling pathways [36–38]. Unlike mammals, *C. elegans* cannot synthesize cholesterol and relies on dietary cholesterol for its normal development and functions. Mammals, on the other hand, require the dietary intake of two polyunsaturated fatty acids, linoleic acid and linolenic acid, in order to synthesize more complex lipids, such as arachidonic acid [39].

Since lipids are a great source of energy, they often need to be stored for an extended period of time. Neutral lipids, such as TAG, are predominantly stored in a conserved organelle called a lipid droplet (LD). LDs are formed when there is a localized accumulation of TAGs within the lipid bilayer of the endoplasmic reticulum (ER), leading to the eventual budding off of a LD [40]. They are surrounded by a phospholipid monolayer, structural proteins called perilipins that protect the LD from cytoplasmic lipases, and other proteins involved in multiple aspects of LD biology, including lipases for lipid degradation/mobilization [41]. Interestingly, *C. elegans* lack perilipins, but still maintain a tight control over LD degradation [42]. Active on-going researches in different laboratories are addressing the fundamental mechanisms underlying LD maintenance and dynamics in *C. elegans*.

In order to metabolize the lipids stored within LDs, two pathways are used, lipolysis and lipophagy (Fig. 14.2). In lipolysis, cytoplasmic and LD-associated lipases degrade the neutral lipids within the LDs. First, ATGL cleaves TAG to generate DAG and a FFA. The DAG can then be degraded by hormone sensitive lipase to generate monoacylglycerol (MAG) and another FFA. MAG can then be degraded by MAG lipase to generate a FFA and glycerol [40]. In *C. elegans*, ATGL-1, which is localized to LDs, is the lipase necessary for lipolysis [42]. In lipophagy, a branch of autophagy, autophagosomes are used to mobilize the lipids stored within LDs. In normal autophagy, cellular contents are engulfed by the autophagosomes that will fuse with lysosomes, resulting in the degradation of the autolysosomal contents and

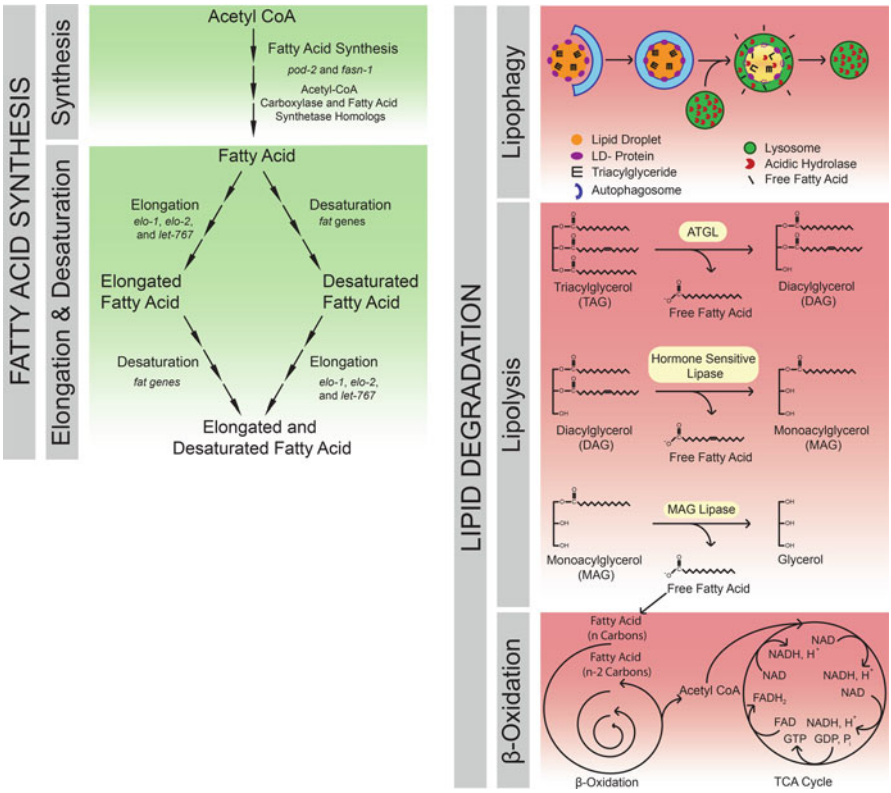

Fig. 14.2 Lipid synthesis, storage and degradation. Fatty acid *de novo* synthesis begins with the extension of acetyl CoA to malonyl CoA by the acetyl CoA carboxylase (POD-2), which is the rate-limiting step of fatty acid synthesis. Malonyl CoA is then extended by fatty acid synthetase into a 16 carbon saturated fatty acid, palmitic acid. Palmitic acid can then be elongated by the elongases (ELO-1, ELO-2, and LET-767) and/or desaturated by the desaturases (FAT-1 to 7). These fatty acids can then be used to synthesize more complex lipids, such as glycerolipids, glycerophospholipids and sphingolipids. These complex lipids are often degraded via specific enzymes. Triacylglycerides, for example, are degraded via two different mechanisms: lipophagy and lipolysis. In lipophagy, all or part of a lipid droplet is engulfed by an autophagosome, which then fuses with a lysosome, resulting in the degradation of triacylglycerols and the release of free fatty acids. In lipolysis, specific enzymes sequentially remove fatty acids from triacylglycerols. Once free fatty acids have been generated by lipolysis or lipophagy, they are cyclically shortened by two carbons via β-oxidation. This results in the production of acetyl CoA, which can then enter the citric acid cycle to generate the reduced electron carrier proteins used by the electron transport chain, NADH and $FADH_2$

subsequent release of building blocks, such as amino acids and FFAs. In lipophagy, LDs are targeted and either completely or partially engulfed by the autophagosomes [43]. The LD-filled autophagosomes then fuse with lysosomes, resulting in the degradation of LDs and subsequent release of FFAs, which are then further degraded by β-oxidation.

β-oxidation occurs via the same reactions in both mitochondria and peroxisomes (Fig. 14.2) [44], but the identity of the enzymes used in these reactions are different between the two organelles [45]. Accordingly, while most fatty acids can be degraded by both organelles, certain fatty acids, such as very long chain fatty acids, prefer peroxisomal degradation [45]. In β-oxidation, the hydrocarbon tails of saturated FFAs are cyclically degraded by two carbons at a time to generate acetyl CoA [44], which is utilized to generate energy via the TCA cycle and oxidized electron carriers, which can be used in the electron transport chain (ETC). Unsaturated fatty acids require additional enzymes, such as isomerases and dehydrogenases, to process the double bonds before the β-oxidation pathway can degrade the fatty acids [46].

14.2.3 Methods of Studying Lipid Metabolism

Since lipids play such a variety of vital roles in cellular homeostasis and organismal fitness, it is important that we have effective methods to study their storage, composition and distribution. In *C. elegans*, lipids are stored in the intestine, the hypodermis and oocytes. The intestine of *C. elegans* provides the function of multiple organs/tissues, such as digestion like the mammalian intestine, detoxification like the liver, and fat storage like the adipose tissue [47]. Additionally, lipids are synthesized in the intestine and transported to oocytes by vitellogenin proteins, where they play major roles in oocyte and embryo development [38, 48, 49].

Biochemical assays are powerful methods to study lipid levels and composition. These can provide knowledge about the relative amounts of different lipid species within a sample, which can be important when examining lipid metabolism at the molecular level. There are two methods that are commonly used to analyse lipids biochemically, mass spectrometry (MS) [3] and nuclear magnetic resonance (NMR) spectroscopy [50]. MS analyses the mass/charge ratio of the molecules in a sample which have to be ionized prior to analysis [51]. Before the samples are analysed via MS, a separation technique, such as gas chromatography, liquid chromatography or capillary electrophoresis, is often performed. The samples are then ionized through one of several techniques. Two of the more common techniques are electron-spray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). These charged molecules will then be separated based upon their mass-to-charge ratio via techniques such as time-of-flight or ion traps, before reaching a detector. Each lipid molecule will give specific peaks in the MS spectrum, which can be detected and analysed.

NMR is based upon the magnetic properties of hydrogens in the compounds, which can be affected by the bonds and connected structures near the hydrogens. This can help to provide physical and chemical details about the lipids analysed [52]. As with every analysis, there are pros and cons for both NMR and MS. Even though MS is more sensitive than NMR [51], NMR is quantitative and does not require extra sample preparation steps. NMR is also a non-destructive technique

where the sample can be recovered and used for further analyses. MS is a destructive technique but requires a much smaller amount of sample than NMR needs [53]. Even though these techniques are useful for detecting different kinds of lipid species at the molecular level, they lack spatial information of lipid distribution.

The cellular/tissue distribution of lipids and their transportation between cells/tissues are very crucial for their functions. The transparent nature of *C. elegans* makes it an ideal model for visualizing lipid storage with subcellular resolution at the whole organism level. There are several stains that are commonly used to visualize the lipid stores of *C. elegans*. Two of these, BODIPY-labelled fatty acids and Nile Red can emit fluorescence when labelling LDs; while two other stains, Sudan Black and Oil Red O, appear blue-black and red colour respectively when enriched in LDs [54]. However, both fluorescent and non-fluorescent-based methods require fixation, and are commonly associated with a higher degree of variability.

Alternative to the staining techniques, chemical imaging methods are established in several laboratories for visualizing lipid species and different metabolites [20, 55–59]. These two, relatively new methods are coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopy, both of which are based upon stimulated Raman scattering. In both of these methods, chemical bonds are stimulated with two lasers, one of which is fixed at a certain wavelength, while the wavelength of the other can be adjusted accordingly to the vibrational frequency of the chemical bond of interest. If the frequency difference of the laser beams matches the vibrational frequency of the chemical bond, the molecular vibration transitions to an excited state. As a result, anti-Stokes signals are emitted and the beam intensities will change, which can be detected and quantified as a measure for the level of the chemical bond of interest [60]. CARS was first demonstrated in 1982 as a viable microscopy method, but was not really used until 1999 [61]. Later, in 2008, SRS was shown to be an improvement over CARS by reducing the non-resonant background, providing easier quantification [20], and quicker, more sensitive imaging [60]. SRS microscopy can visualize lipid storage at diffraction-limited spatial resolution and with 3D imaging capacity in living cells and organisms [62]. More recently, by administering deuterium-labelled [63] or alkyne-labelled [64] lipids to *C. elegans* or mammalian cells and using SRS microscopy to detect the specific signals from these labels, this technique was proven to be useful also for analysing the incorporation, synthesis and degradation of lipids.

14.3 Pathways Regulating Ageing

In 1993, the discovery that loss-of-function mutations in *daf-2* could double the lifespan of *C. elegans* accelerated the field of ageing research. Since then, considerable effort has been put into elucidating the genetic pathways involved in the regulation of organism ageing and longevity. These pathways often have common components and crosstalk. For example, *nhr-80* is a downstream effector for both *glp-1* and *lipl-4*. In this part, we are going to discuss the important longevity regulating signalling pathways in *C. elegans* and their effects on lipid metabolism.

14.3.1 Insulin/IGF-1 Signalling (IIS)

daf-2 is a key player in the insulin/IGF-1 signalling (IIS) pathway and its role in ageing is discussed in Chap. 4. It encodes the *C. elegans* homologue of the insulin/IGF-1 receptor [2], which is a receptor tyrosine kinase. When bound to an activating ligand, the DAF-2 receptor activates AGE-1, the *C. elegans* homologue of phosphoinositide 3-kinase (PI3K). AGE-1/PI3K then phosphorylates phosphatidylinositol 4,5-bisphosphate to generate phosphatidylinositol 3,4,5-trisphosphate, which then activates a kinase cascade culminating in the phosphorylation of multiple proteins, including DAF-16/FoxO [65]. DAF-16/FoxO is a transcription factor and when phosphorylated as a result of the active IIS, it is sequestered in the cytoplasm along with several other transcription factors [65]. When IIS is low, DAF-16/FoxO translocates to the nucleus and promotes longevity by regulating the expression of genes involved in biological processes including both fat metabolism and ageing [66, 67].

There are a number of *daf-2* mutant alleles that lead to lifespan extension [68]. In addition to the longevity phenotype, mutants with the hypomorphic *daf-2(e1370)* allele, have increased lipid storage as shown by Nile Red, Oil Red O staining [69] and CARS/SRS microscopy analyses [20], and elevated *de novo* lipid synthesis assayed by ^{13}C isotope labelling strategy [3]. However, different lifespan-extending alleles of *daf-2* can have different effects on lipid synthesis and storage. For example, the *m577* and *e1368* alleles showed no increase in *de novo* lipid synthesis or total lipid storage [3]. This has lead to more detailed analyses of *daf-2* mutants at the transcriptional, metabolic and protein levels. The *daf-2(m21)* mutant was found to downregulate the expression of several of *vit/* lipid transport genes and upregulate several *fat/* fatty acid desaturase genes [70]. Proteomics analysis of the *daf-2(e1370)* mutant also revealed that intermediary metabolism is reorganized, and some of these changes might be related to increased lipid storage and longevity in this mutant allele [71]. Furthermore, several *daf-2* alleles were also subjected to metabolite profiling, and amongst a variety of metabolite changes, choline metabolism was specifically reprogrammed, possibly due to altered phospholipid metabolism [72].

14.3.2 Dietary Restriction

eat-2 encodes a non-α-nicotinic acetylcholine receptor subunit [73]. It is expressed in the pharyngeal muscle and required for the proper neuromuscular junction (NMJ) activity, specifically the NMJ with the MC neuron [74]. Because of irregular and slow pharyngeal pumping in *eat-2* mutants [74], they have been utilized to model caloric restriction, show a lifespan extension of 29–57 % [75], and display decreased lipid storage as shown by several visualization methods [19] and enzymatic assays [76, 77]. *eat-2* longevity, decreased lipid storage, and increased autophagy are all dependent upon *nhr-62*, which encodes a nuclear hormone receptor [4]. In addition

to being required for *eat-2* longevity, NHR-62 has been shown to play a role in other forms of dietary restriction, such as using a diluted bacterial diet [4]. A more detailed discussion of dietary restriction can be found in Chap. 16.

14.3.3 Germline Loss

In various species, germline signals have been linked with longevity regulation [78–80]. In *C. elegans*, ablation of germline precursor cells leads to more than 50 % lifespan extension [81], and is discussed in detail in Chap. 6. Similar longevity phenotypes were also observed in the loss-of-function mutant of *glp-1*, which lacks germline stem cells [82]. *glp-1* encodes one of the two DSL-family Notch receptors, is expressed in germline stem cells, perceives signals from their niche provided by the distal tip cells, and is required to maintain the germline stem cell pool [82, 83]. Beside its longevity phenotype, the *glp-1* mutant also displays increased lipid storage, as shown by Oil Red O staining, MS analysis [54], and CARS microscopy [19]. This intestinal lipid accumulation is thought to be largely due to absence of lipid transfer from the intestine to oocytes when germline development is arrested. Several factors have been implicated in the regulation of the longevity conferred by germline deficiency, including the nuclear receptors, *daf-12, nhr-49* and *nhr-80*, the transcription factor, *daf-16,* and the lipase, *lipl-4.*

1. ***daf-12*** encodes a nuclear receptor that is required for the longevity conferred by removal of germline stem cells. DAF-12 binds to the endogenous cholesterol derivatives $\Delta^{1,7}$-dafachronic acid (DA), Δ^{7}-DA and 3α-OH-Δ^{7}-DA [50], and regulates the expression of a variety of genes involved in development and metabolism [84, 85]. Gain-of-function alleles of *daf-12* occur in the ligand-binding domain of the protein, resulting in increased activity and lifespan extension [86]. On the other hand, *daf-12* loss-of-function alleles are mostly in the DNA binding domain, and show shortened lifespans. Knockdown of *daf-12* results in a slight decrease in lipid levels [87], but gain-of-function alleles of *daf-12* display increased fat content (unpublished results). DAF-12 functions with the corepressor DIN-1S, which regulates lipid storage [88]. *daf-12* knockdown affects the expression of several lipid metabolic genes, such as *lipl-4*, *lips-17*, and *fard-1*, and these genes are required for the longevity phenotype of the *glp-1* mutant [89].
2. ***nhr-80*** encodes a nuclear hormone receptor that is also required for the longevity phenotype of *glp-1* mutants [90]. Once bound to its ligand, NHR-80 can use other nuclear receptors, such as NHR-49 and DAF-12, as cofactors to regulate the expression of its target genes, which include lipid metabolic genes such as *acs-2* and *fat-6* [91, 92]. Loss of *nhr-80* function does not affect the lifespan of wild type animals, but completely abrogates the lifespan extension of the *glp-1* mutant [90]. *nhr-80* mutants have no changes in their total amount of lipids stores, but do display changes in the relative lipid composition of their lipid stores [91, 92].

3. ***nhr-49*** encodes a nuclear hormone receptor necessary for β-oxidation gene expression [93]. *nhr-49* is required for the increased lipid accumulation and lifespan extension in the *glp-1* mutant [93], and also plays a crucial role in executing adult reproductive diapause (ARD) in which adults halt reproduction and survive for an exceptionally long time [94]. Mutations in *nhr-49* affect *de novo* lipid synthesis and consequently lipid storage, at least in part via altering expression of several fatty acid desaturase genes [93].
4. ***lipl-4*** encodes a lysosomal triglyceride lipase that is induced in the *glp-1* mutant and required for its longevity [87]. When constitutively over expressed in the intestine, *lipl-4* is sufficient to extend lifespan on its own [10, 87]. Interestingly, the *lipl-4*-induced longevity effect requires both NHR-49 and NHR-80. Metabolite profiling of the long-lived *lipl-4* transgenic worms revealed the induction of several lipid molecules; amongst them, a specific fatty acid derivative OEA acts as an agonist of the nuclear receptor NHR-80, and is sufficient to prolong lifespan when supplemented to wild type worms [10, 87]. The transduction of lysosome-to-nucleus lipid messenger signalling requires a specific fatty acid binding protein LBP-8 [10].

14.4 Lipids as Signalling Molecules

Beside their well-known functions as energy fuels and structural building blocks, lipids play important roles in both intracellular and extracellular signalling. While some of these signalling functions have been thoroughly established, others are still being discovered and elucidated. Emerging studies have revealed the significance of signalling lipid molecules in the regulation of organism longevity, and have discovered the involvement of protein chaperones, transporters and receptors in shuttling lipid molecules between compartments, as well as recognizing and transducing lipid signals.

14.4.1 Lipid Messengers

In *C. elegans,* a variety of lipids have signalling capabilities. One group of external lipid-derived signals are the ascosaride dauer-inducing pheromones [95]. These "daumones" are secreted by *C. elegans* into the environment and induce dauer formation in nearby larvae by binding to and activating cell surface receptors on ciliated chemosensory neurons. Each daumone has a different ability to induce dauer formation [96], and they often function together [97].

Beyond external lipid signals like daumones, *C. elegans* uses several internally generated lipid signals to communicate within and between cells. For example, the inositol-signalling pathway, a well-conserved intracellular signalling pathway, is involved in a variety of processes, including embryonic development [98] and life-span

[99]. Additionally, free fatty acids and their derivatives play roles in proper neurotransmission [100] and lifespan [10], amongst other processes.

Last but not least, sterols play an important role in biological signalling. Sterols, which include cholesterol and its derivatives, are lipids with four carbon rings and auxiliary components. Cholesterol is the basis for many of the hormones used in mammals, and plays a major role in *C. elegans* biology. Sterols cannot be generated *de novo* in *C. elegans*, so their dietary inputs play a key role in the evaluation of environmental quality. A key transcription factor, SBP-1, the conserved homologue of the mammalian sterol regulatory element binding protein, SREBP-1, controls the expression of several of the *fat*/fatty acid desaturase genes, along with other fatty acid synthesis genes [34, 101]. However, it is not known whether SBP-1 also regulates sterol metabolism in *C. elegans* as SREBP-1 does in mammals.

14.4.2 Proteins as Lipid Signalling Chaperones

There are several lipid-binding chaperones in *C. elegans*, and two major families are lipid-binding proteins (LBPs) and vitellogenins. There are 9 *lbp* genes in *C. elegans*, which have varied tissue and developmental expression patterns. Three of these, *lbp-1*, *lbp-2*, and *lbp-3*, have secretory signals suggesting a role in extracellular signalling [102]. Other LBPs play important roles in intracellular signalling. For example, *LBP-5* is involved in multiple aspects of metabolism, such as β–oxidation, fat storage, and glycolysis [103], and *LBP-8* mediates lysosome-to-nucleus communication [10]. The other family of lipid-binding chaperones is the yolk proteins encoded by 6 vitellogenin, or *vit* genes [104]. These yolk proteins are produced exclusively in the intestine of hermaphrodites [105] and function to transport lipids from the intestine to oocytes.

Both families of lipid-binding chaperones have been linked to lifespan regulation. For example, LBP-8 was recently shown to shuttle OEA from the lysosome to nucleus where OEA binds to and activates NHR-80. When *lbp-8* is overexpressed, the increased shuttling of OEA and subsequent activation of NHR-80 results in a longer lifespan [10]. Additionally, when knocked down, *vit-5* results in lifespan extension [106], and in long-lived *daf-2* mutants, all six of the *vit* genes are down regulated [70].

14.4.3 Lipid Signalling Receptors

As signalling molecules, lipids can bind to and activate G-protein coupled receptors (GPCRs) and nuclear hormone receptors (NHRs). There are almost 2000 GPCRs in *C. elegans* most of which are expressed in individual ciliated chemosensory neurons to sense their environmental cues [107], which includes the lipid derivatives,

daumones [108]. Several GPCRs are required for the daumone-induced dauer-formation response, such as SRBC-64/SRBC-66 [109], SRG-36/SRG-37 [110], and DAF-37/DAF-38 [111]. One of these receptors, DAF-37, is specific for the ascaroside#2, but can mediate different responses to ascaroside#2 depending upon if DAF-37 is activated in the ASK or ASI chemosensory neurons [111].

C. elegans have 284 NHRs, which are transcription factors with a DNA-binding domain and a ligand-binding domain that is often activated by small hydrophobic molecules such as lipids and lipid derivatives [50, 112]. Several NHRs regulate the expression of genes important in lipid metabolism and/or longevity pathways, such as DAF-12 in the germline regulation of lifespan [81], NHR-49 in lipid metabolism, lipid storage, and lifespan [113], NHR-80 in germline-mediated longevity and the associated lipid metabolism and storage changes [90], and NHR-62 in caloric restriction-induced longevity [4]. These receptors respond to signals that are usually generated within the organism. For example, DAF-12 is activated by three endogenous derivatives of dafachronic acid (DA), $\Delta^{1,7}$-DA, Δ^{7}-DA and 3α-OH-Δ^{7}-DA [50], and NHR-80 is activated by the endogenous fatty acid derivative OEA (*10*).

14.5 Relevance to Humans

Since *C. elegans* is a eutelic nematode roughly the size of a comma in a sentence, it is often difficult to realize how studies in *C. elegans* can be relevant for human biology. *C. elegans* and mammals appear vastly different, in no small part due to their anatomical and physiological differences. Despite these glaring differences, most of the proteins and genes mentioned throughout this chapter are not only functionally conserved, but are often structurally conserved as well [114, 115].

When studying human ageing, some populations have been especially valuable when looking for insights into healthy ageing. One especially important population are the centenarians. Multiple studies have been performed to look for the genes that may explain their longer life. Not surprisingly, hypomorphic mutations in the gene IGF1R, which encodes the insulin-like growth factor 1 receptor, has been implicated in the longevity displayed by some centenarians [116]. Variants in other components of the IIS pathways, such as INSR [117], PI3K [117], AKT1 [118] and FOXO3A [118–122] have been examined in several populations, and are associated with longevity as well.

Additionally, multiple studies have been performed to study the centenarian people from Okinawa, Japan since it was first noticed in the 1960s that they were unintentionally calorically restricting themselves [123, 124]. Although there have been many studies on the effects of relatively short-term caloric restriction on a variety of ageing hallmarks [125–127], this population has provided the best evidence for the conservation of many benefits of caloric restriction.

14.6 Conclusion and Perspectives

As we age, changes in our metabolism occur. One noticeable change is in our lipid metabolism, especially the localization and quantity of lipid storage. These storage locations respond to ageing and disease states differently. As we age, the subcutaneous storage of fat tends to decrease, but visceral fat tends to remain and increase [128]. The accumulation of visceral fat is associated with several disease states, such as insulin resistance and cardiovascular disease [129]. In addition to the proportion of fat stored in visceral fat in aged individuals being important for healthfulness, it is becoming more evident that the profile of the lipids stored is important as well. In some neurodegenerative diseases, such as Alzheimer's, the metabolism of certain lipid species, such as arachidonic acid, has been shown to be altered, which may play a role in the progression of the disease [130]. In obese people, the rate of fat breakdown is decreased. Relatedly, lipid turnover is also decreased and inversely correlated with insulin resistance in both obese people and people with familial combined hyperlipidemia [131]. This points to the importance not just of the composition and tissue distribution of lipids, but also how long they are stored.

Hopefully key insights will be gained into the role of individual lipids/lipid species in modulating ageing, the differences between subcutaneous and visceral fat that leads to both decreased subcutaneous fat storage and increased insulin resistance, the molecular mechanism behind lipid turnover's relationship with insulin resistance, and more. Using animal models such as *C. elegans*, which have multiple lipid storage tissues, will end up being a critical aspect of this future knowledge.

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