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Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of Fads and Elovl gene repertoire

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Abbreviations

aa, amino acid

ACP, acyl carrier protein

ALA, α-linolenic acid (18:3n-3)

ARA, arachidonic acid (20:4n-6)

BHT, butylated hydroxytoluene

cDNA, complementary DNA

CoA, Coenzyme A

DHA, docosahexaenoic acid (22:6n-3)

ELOVL, elongation of very long-chain fatty acid protein

EPA, eicosapentaenoic acid (20:5n-3)

ER, endoplasmic reticulum

FACES, fatty acid chain elongation system

FADS, fatty acyl desaturase

FAE1, fatty acid elongase 1

FAS, fatty acid synthase

HADC, β-hydroxyacyl-CoA dehydrase

KAR, β-ketoacyl-CoA reductase

KCS, β-ketoacyl-CoA synthase

LA, linoleic acid (18:2n-6)

LC-PUFA, long-chain (C_{20-24}) polyunsaturated fatty acids

ORF, open reading frame

PKS, polyketide synthase

PUFA, Polyunsaturated fatty acid

SCD, stearoyl-CoA desaturase

TER, trans-2-enoyl-CoA reductase

VLC-PUFA, very long-chain ($>C_{24}$) polyunsaturated fatty acid

WGD, whole genome duplication

Abstract

Long-chain polyunsaturated fatty acids (LC-PUFA) are major components of complex lipid molecules and are also involved in numerous critical biological processes. Studies conducted mainly in vertebrates have demonstrated that LC-PUFA can be biosynthesized through the concerted action of two sets of enzymes, namely fatty acyl desaturases (Fads) and elongation of very long-chain fatty acid (Elovl) proteins. While LC-PUFA research is a thriving field, mainly focused on human health, an integrated view regarding the evolution of LC-PUFA biosynthetic genetic machinery in chordates is yet to be produced. Particularly important is to understand whether lineage specific life history trajectories, as well as major biological transitions, or particular genomic processes such as genome duplications have impacted the evolution of LC-PUFA biosynthetic pathways. Here we review the gene repertoire of Fads and Elovl in chordate genomes and the diversity of substrate specificities acquired during evolution. We take advantage of the magnitude of genomic and functional data to show that combination duplication processes and functional plasticity have generated a wide diversity of physiological capacities in extant lineages. A clear evolutionary framework is provided, which will be instrumental for the full clarification of functional capacities between the various vertebrate groups.

Keywords

Chordates; elongation of very long-chain fatty acid protein; evolution; front-end desaturase; long-chain polyunsaturated fatty acids

1. Introduction

Unlike proteins and carbohydrates, that generally have structures based on long chains (polymers) of amino acid (polypeptides) or sugar (polysaccharides) residues, lipids are a much more diverse range of compounds with considerable variations in structure. However, although lipids do not have "building blocks" as such, fatty acids come closest, being components of many lipid classes including acylglycerols (glycerides and phosphoglycerides) and sphingolipids. In complex lipids, fatty acids are esterified to alcohol or amino groups and, as these lipid classes are the predominant forms, fatty acids constitute the bulk of lipid. All fatty acids play important roles in key biological processes including energy supply, structure and functions of biological membranes. Some fatty acids, particularly polyunsaturated fatty acids (PUFA) and their derivatives, are highly biologically active and involved in signalling and the regulation of lipid metabolism, inflammatory response and cell division [1]. Saturated and monounsaturated fatty acids can be biosynthesized by all organisms whereas PUFA generally have to be obtained in the diet of animals although they can be converted to long-chain (C_{20-24}) PUFA (LC-PUFA) in some species. This review will focus on two sets of enzymes, fatty acyl desaturases and elongases that participate in the biosynthesis of LC-PUFA in chordates. In particular, we will review the currently available data on the desaturase and elongase gene[1] repertoire present in chordate genomes and the diversity of substrate specificities that have been acquired by the encoded enzyme proteins during evolution. For clarity purposes, we will first provide a description of the fatty acid nomenclature system used in this paper, as well as a definition of the groups of organisms that compose the chordate phylum.

[1] **Gene/protein nomenclature**

The standard vertebrate gene symbol formatting determines that different conventions apply to name gene/protein in different model organisms including human (*Homo sapiens*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), chicken (*Gallus gallus*), Carolina anole (*Anolis carolinensis*), frog (*Xenopus laevis* or *X. tropicalis*) and zebrafish (*Danio rerio*). Using as example "Elovl5", the human gene is referred as "*ELOVL5*" and the predicted protein as "ELOVL5"; for mouse and rat, gene will be named as "*Elovl5*", whereas protein will be "ELOVL5"; for chicken and other birds, gene will be termed as "*ELOVL5*", whereas protein will be "ELOVL5"; for anole and other reptiles, gene will be termed as "*elovl5*", whereas protein will be "ELOVL5"; for frog and other amphibians, gene will be named as "*elovl5*", whereas protein will be "Elovl5"; similarly, for zebrafish and other fish, gene will be named as "*elovl5*", whereas protein will be "Elovl5". For non-vertebrate organisms and agnathans, we have used the same symbols as described for fish ("*elovl5*" for genes and "Elovl5" for proteins).

1.1 Nomenclature and structure of fatty acids

A fatty acid is essentially an organic molecule with a carboxylic acid group at the end of an aliphatic chain containing four or more carbons, usually an even number up to 24, although odd-numbered and longer carbon chains are also found (Fig. 1). The aliphatic chain can be "saturated", where all carbon-carbon linkages are single bonds and all other carbon bonds are taken by hydrogen, or "unsaturated", where some carbons are linked by double bonds. Several systems have been used historically for fatty acid nomenclature, but the most commonly used and internationally accepted is that defined by the International Union of Pure and Applied Chemistry (IUPAC) in the Compendium of Chemical Terminology (IUPAC, 1997). In the n-x (or "omega x") system of nomenclature, fatty acids are described by the general formula, C:Dn-x, where C = chain length, D = number of ethylenic/double bonds, and n-x (or ωx) indicates the position of the first double bond relative to the methyl end of the chain. Therefore, in this nomenclature 18:0 represents a saturated fatty acid containing an 18-carbon aliphatic chain with no double bonds, and 18:1n-9 (18:1ω9) denotes a monounsaturated fatty acid with an 18-carbon aliphatic chain with a single, *cis* double bond 9 carbons from the methyl group. Polyunsaturated fatty acids (PUFA), any fatty acid containing two or more double bonds that are most commonly separated by methylene (CH_2) groups, are represented as in the following example, 20:5n-3 (20:5ω3), which denotes a 20-carbon aliphatic chain containing five double bonds with the first situated three carbons from the methyl group (Fig. 1). However, in the present review, the main alternative nomenclature, the Δx (delta-x) system, is equally important as this is the one commonly used for specifying activities of the fatty acyl desaturase (Fads) enzymes studied herein. In this nomenclature the double bonds are numbered from the carboxyl end of the molecule and so 20:5n-3 is written as $20{:}5^{\Delta5,8,11,14,17}$. Thus, a Fads that introduces an ethylenic (double) bond five carbons from the carboxyl end of the aliphatic chain is described as having Δ5 activity. Additionally, fatty acids are often still described using trivial names, often reflecting their main sources, such as palmitic acid (16:0) and oleic acid (18:1n-9) from palm and olive oils, respectively. Semi-systematic names, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are more useful as they at least indicate the numbers of carbons (e.g. eicosa-, 20) and double bonds (e.g. pentaenoic, 5). It is important to clarify that, while PUFA applies to any fatty acid with at least two double bonds and generally, with chain lengths from 18 carbons or more, we will use the term long-chain PUFA (LC-PUFA) for fatty acids with an aliphatic chain length from C_{20} to C_{24}, and two or more double bonds. Similarly, very long-

chain PUFA (VLC-PUFA) refer to PUFA with two or more double bonds, with fatty acyl chains > C_{24}.

1.2 Classification of chordates

Metazoans, a classification that refers to animals, are characterized by being multicellular and heterotrophic, possessing epithelial cells and having the ability to produce sperm and eggs. Their overwhelming diversity can be organized into defined groups on the basis of phylogenetic relationships. The first major division separates two clades on the basis of their body plan symmetry: the pre-Bilateria (e.g. sponges, coral and jellyfish) and the Bilateria. Within the latter, two other groups can be distinguished based on early embryonic development features: the protostomes (e.g. molluscs) and the deuterostomes (e.g. sea urchins, mammals). In this review we will focus primarily on the phylum chordates that, together with hemichordates and echinoderms, comprise the deuterostomes. Chordates are subdivided into three sub-phyla, namely cephalochordates, tunicates and vertebrates (Fig. 2). Cephalochordates and tunicates are usually referred to as "invertebrate chordates" as they lack vertebrae (Fig. 2). Chordates share a number of characteristics including a dorsal hollow nerve cord, pharyngeal slits, postanal tail and a prominent axial notochord. Over the years a consensus over the phylogenetic relationships between these three lineages emerged, with tunicates now considered the sister clade of the vertebrates [2]. The transition towards vertebrates entailed a significant number of innovations including the presence of an endoskeleton, elaborated brain and distinct head [3]. A fundamental separation within the vertebrates involves the presence or absence of a jaw, which divides the agnathans (lampreys and hagfishes), which lack a jaw, from gnathostomes (cartilaginous fish, teleosts, amphibians, birds and reptiles and mammals) (Fig. 2).

Comparative studies involving species from key phylogenetic lineages within the chordates have shed light over the specific acquisition (and loss) of numerous developmental, morphological and physiological traits [3-5]. An underscoring aspect of this research is the impact of gene duplication, particularly that arising from whole genome duplications (WGD). It is clear today that WGD events took place in early vertebrate evolution, although the number of events and exact timing remains contentious (Fig. 2) [6-9]. Nevertheless, the increment of gene numbers followed by episodes of sub-functionalizations, neofunctionalizations, and gene loss, have all impacted vertebrate physiology and, with regard to the topic of this review, the number and function of genes encoding key enzymes

involved in the biosynthesis of LC-PUFA. Vertebrates have a surprising diversity of species denoting a wide range of adaptations. They also colonize a variety of habitats from aquatic (fresh and salt) to land and air, which have hinted at impacts on lipid metabolism [10]. In the context of the present review, the availability of full genome sequences in species representatives of the main lineages clearly provides the necessary tools to address the evolutionary and functional aspects of LC-PUFA molecular components (Fig. 2).

2. Biosynthesis of fatty acids

2.1. Biosynthesis of saturated fatty acids

The lipogenic pathways are essentially the same in plants and animals including vertebrates although most studies in animals have been conducted in mammals. The primary pathway of lipogenesis is the biosynthesis of fatty acids, which is catalyzed by two cytosolic enzyme systems, acetyl-CoA carboxylase and the multienzyme fatty acid synthase (FAS) complex that uses acetyl-CoA as carbon source to produce saturated fatty acids, primarily 16:0 in animals and 18:0 in plants [11]. There are two main classes of FAS; Type I systems as found in yeast and animals use a single large, multifunctional polypeptide whereas Type II systems that are found in prokaryotes and plants utilize a series of discrete, monofunctional enzymes. The FAS mechanism consists of sequential decarboxylative Claisen condensation reactions with the key step being the condensation of malonyl-CoA, formed by acetyl-CoA carboxylase, with the growing acyl chain. After each round of elongation the β-keto group is reduced to the fully saturated carbon chain by the sequential action of ketoacyl reductase, dehydrase, and enoyl reductase activities. The growing acyl chain is carried between the enzyme active sites by the acyl carrier protein (ACP) domain through a covalent linkage to the phosphopantetheine prosthetic group, and is finally released as 16:0 by the action of a thioesterase. Acetyl-CoA is initially produced in mitochondria from both carbohydrate or protein sources through the oxidative decarboxylation of pyruvate or the oxidative catabolism of some amino acids, respectively, leading to citrate production (via the tri-carboxylic acid cycle) and export to the cytosol, and subsequent production of cytosolic acetyl-CoA through the action of ATP-citrate-lyase. The NADPH reducing equivalents are produced by enzymes of carbohydrate metabolism including the pentose phosphate pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase), tricarboxylic acid cycle (NADP-dependent isocitrate dehydrogenase) and malic enzyme.

The FAS complexes are very similar to another family of multi-enzyme complexes, the polyketide synthases (PKS), that use a similar mechanism and homologous domains to produce structurally complex organic molecules or secondary metabolites, collectively termed polyketides, in bacteria, fungi, and plants [12]. Both FAS and PKS have two principal classes, Type I and Type II, with the differences between PKS Types being the same as described above for FAS, e.g. Type II being characterized by discrete, monofunctional enzymes. The relationship between FAS and PKS is particularly interesting and relevant to the present review for two main reasons. Firstly, the evolutionary history of FAS is intimately linked with that of PKS as the Type I FAS in animals is believed to have evolved by modification of fungal Type I PKS, whereas Type I FAS in fungi (and some bacteria) appears to have arisen through the fusion of Type II FAS genes. Secondly, LC-PUFA including EPA and DHA may be biosynthesized by PKS-like pathways (see below) in some marine microalgae including some dinoflagellates and thraustochytrids [13].

2.2 Fatty acid chain elongation

Although FAS produces 16:0 and 18:0, C_{20} - C_{24} fatty acids are common and chain lengths > C_{24} are often found in eukaryotes [14, 15]. Therefore, fatty acid elongation is another major pathway in the production of fatty acids. Depending upon the organism, different terms such as “elongase” or “elongase system”, or fatty acid chain elongation system (FACES) have all been used to describe enzymes responsible for the elongation of fatty acids through the addition of two carbon units to the carboxyl end of the chain. In higher plants, elongation is usually restricted to saturated and monounsaturated fatty acids and accomplished by an enzyme coded by the FAE1 gene (Fatty acid elongase 1) [16], although a PUFA Fae1 has been functionally characterized in the marine parasitic protozoon *Perkinus marinus* [17]. Marine microalgae rich in n-3 LC-PUFA appear to have an elongase (e.g IgASE1 in *Isochrysis galbana*) capable of elongating PUFA [18]. Similarly, marine protists such as thraustochytrids may also have an elongase capable of elongating PUFA [19]. The yeast, *Saccharomyces cerevisiae*, only produces saturated and monounsaturated fatty acids and has a family of elongases, ELO1, ELO2 and ELO3, with different fatty acid specificities according to chain length [19, 20]. The PUFA-producing fungus, *Mortierella alpina*, that produces arachidonic acid (ARA; 20:4n-6) has two elongases including GLELO that elongates 18:3n-6 to 20:3n-6 and is likely the rate-limiting step in ARA biosynthesis, and MAELO that preferentially elongates saturated and monounsaturated fatty acids [21].

In plants and animals, the elongation of fatty acids to produce fatty acids with chain lengths > C_{18} involves four sequential reactions to add each 2-carbon unit that occurs in endoplasmic reticulum (ER) [19, 22] (Fig. 3). These reactions are analogous to those of *de novo* synthesis, (specifically condensation, 1st reduction, dehydration and 2nd reduction) and are catalyzed by four membrane-bound enzymes, namely Elongation of very long fatty acids (Elovl) proteins (condensing enzyme), β-ketoacyl-CoA reductase (Kar; 1st reduction), β-hydroxyacyl-CoA dehydrase (Hadc; dehydration), and trans-2-enoyl-CoA reductase (Ter; 2nd reduction) (Fig. 3). Early biochemical studies provided indirect evidence to indicate that the condensing enzyme Elovl of the elongation system was rate-limiting, regulated the specificity of elongation in term of chain length and the degree of unsaturation, and was regulated by nutritional and hormone status [19]. Cloning of the condensing enzyme and functional characterization of the activity confirmed these data and other enzymes in the complex do not show any fatty acid specificity [20, 23]. While expanded in Section 3, it is worth clarifying that ELOVL enzymes comprise a family with at least seven members, ELOVL1-7, that can be broadly sub-divided into elongases of saturated and monounsaturated fatty acids, ELOVL1, ELOVL3, ELOVL6 and ELOVL7, and elongases of PUFA, ELOVL2, ELOVL4 and ELOVL5 [20, 22, 24]. It is worth clarifying that Elovl and Fae1 are both β-ketoacyl-CoA synthases (Kcs) and, although structurally unrelated, both enzymes have been suggested to similarly interact with the "core" elongase components Kar, Hadc and Ter (Fig. 3) [25]. Interestingly, a new component of the plant elongation pathway, the ECERIFERUM2-like proteins, are believed to affect the Fae1-like Kcs although the mechanism is not yet been established [26]. Preliminary *in silico* analyses appear to suggest that such a paralogue does not exist in non-plant organisms.

2.3 Biosynthesis of unsaturated fatty acids

The biosynthesis of unsaturated fatty acids can occur by two pathways. The first pathway, found in many bacteria, generates the double bonds by essentially leaving those created during the biosynthesis of the fatty acid [27]. Some marine bacteria (e.g. *Shewanella* sp.) are capable of EPA production and analysis of the amino acid sequences derived from the genes responsible showed they were related to microbial PKS (and FAS) complexes [28]. Related genes have been analysed from DHA-producing organisms, the bacterium *Moritella marina* strain MP-1 [29] and *Schizochytrium*, a thraustochytrid marine protist [30]. The second pathway, found in almost all eukaryotes and some bacteria, involves aerobic desaturase enzymes that directly regioselectively introduce double bonds into fatty acids produced by *de*

novo synthesis or obtained through the diet [31, 32]. Although the basic mechanism of the desaturation reaction, removing two hydrogens to create a carbon-to-carbon double bond, is similar and they all utilize molecular oxygen and reducing equivalents obtained from an electron transport chain, fatty acid desaturases have been classified in a number of ways.

2.3.1 Classification of fatty acid desaturases

Probably the most fundamental classification of fatty acid desaturases is based on subcellular location, which classifies fatty acid desaturases into two groups, the soluble desaturases and membrane-bound desaturases, that are phylogenetically unrelated [31]. The only soluble desaturase is the acyl-acyl carrier protein (ACP) or stearoyl-ACP desaturase, found only in the stroma of plant plastids, which is responsible for the production of oleic acid (18:1n-9) from stearic acid (18:0) [33]. In contrast, membrane-bound desaturases are ubiquitous in eukaryotes and bacteria, and are characterized by three histidine box motifs that contain eight histidine residues [34]. The membrane-bound desaturases can be sub-divided based on the nature of the lipid substrate to which the fatty acyl chain is linked [35]. First, acyl-lipid desaturases, which are associated with the ER and chloroplast membrane in plants, cyanobacterial thylakoid membranes, plasma membrane of some bacteria [36], and ER in *Tetrahymena* [37], desaturate fatty acids esterified to glycerolipids. Second, acyl-CoA desaturases, found in the ER of animals and fungi, and plasma membrane of some bacteria, introduce double bonds into fatty acids esterified to coenzyme A (CoA) [38]. A separate classification is based on which end of the fatty acid molecule the desaturase "counts" from in determining specificity. Thus, methyl end desaturases insert the double bond at a fixed number of carbons from the methyl group and are termed ω-desaturases (e.g. ω3 or ω6 desaturases), whereas the so-called "front-end" desaturases insert the double bond as a fixed number of carbons from the carboxyl group and are termed Δ (delta)-desaturases (e.g. Δ6 or Δ5 desaturases). In Section 3, particular attention will be paid to front-end desaturases termed FADS in mammals. The protein sequences of the FADS-like desaturases possess features characteristic of all membrane-bound fatty acid desaturases, including the three histidine boxes mentioned above, two transmembrane regions, and an N-terminal cytochrome b_5 domain containing the haem-binding motif, HPGG [34, 39, 40]. Thus, the animal front-end desaturases are fusion proteins containing both desaturase and cytochrome b_5 functions [41].

2.3.2 Biosynthesis of monounsaturated fatty acids and C_{18} PUFA

Monounsaturated fatty acids, such as 18:1n-9 and 16:1n-7, are produced through the activity of Δ9 fatty acyl desaturases, including the soluble stearoyl-ACP desaturase in plants, and the microsomal stearoyl-CoA desaturases of yeast (OLE1) and animals (SCD) [42-44] (Fig. 4). Two representatives of mammalian SCD have been crystallized and subjected recently to X-ray analysis [45, 46]. Therefore, this activity is ubiquitous and found in all living organisms [47]. In plants and algae, monounsaturated fatty acids can be further desaturated through the action of chloroplast membrane-bound desaturases to form, firstly, linoleic acid (LA; 18:2n-6) from 18:1n-9 and then α-linolenic acid (ALA; 18:3n-3) from LA [42, 48] (Fig. 4). The production of LA is catalyzed by an ω6 desaturase that counts from the pre-existing Δ9 bond (rather than the methyl end) in higher plants, and a Δ12 desaturase in cyanobacteria [43, 49]. The production of ALA from LA is catalyzed by ω3 or Δ15 desaturases [42, 50]. In addition, PUFA can be produced by the action of Δ12 desaturases in some yeast [51] and bifunctional desaturases showing both Δ12 and Δ15 activities have also been described in protozoans [52, 53] and fungi [54].

In contrast to plants, fungi and some aquatic microorganisms, few other organisms are capable of producing PUFA *de novo*. In animals, the Δ12 desaturation of 18:1n-9 to form LA was demonstrated in axenic tissues from insects including cockroach [55] and cricket [56], and insect Δ12 desaturase genes were subsequently isolated and characterized [57, 58]. The nematode, *Caenorhabditis elegans* has also been shown to have two desaturase-like genes, *fat1* and *fat2*. The *fat1* gene encodes a novel ω3 desaturase capable of introducing an ω3 double bond into both C_{18} and C_{20} substrates [59], and the *fat2* gene encodes a functional Δ12 that can desaturate both C_{18} and C_{16} substrates [60], and has been shown recently to also have Δ15 desaturation activity [61]. However, PUFA cannot be synthesized *de novo* by chordates/vertebrates as they lack the necessary Δ12/ω6 and Δ15/ω3 desaturases [38]. The desaturase enzymes involved in the biosynthesis of monounsaturated and C_{18} polyunsaturated fatty acids are important in defining context, however the Δ9, Δ12/ω6 and Δ15/ω3 desaturases are outwith the scope of the present review.

2.4 Biosynthesis of LC-PUFA in plants, bacteria and fungi

De novo biosynthesis of LC-PUFA with chain lengths longer than C_{18} occurs in phototrophic and heterotrophic algae, especially marine microalgae that are rich in EPA and/or DHA, and some marine bacteria. In algae, LC-PUFA can be produced by two

pathways, either the aerobic pathway using specific, discrete desaturase enzyme proteins [18, 51] or, in some cases including certain thraustochytrids and possibly flagellates, anaerobically via polyketide (PKS) pathways [13]. The aerobic pathway synthesises LC-PUFA from the C_{18} PUFA produced through the action of the Δ12/ω6 and ω3 desaturases as described above (Section 2.3). However, the desaturases involved in LC-PUFA biosynthesis are termed "front-end" desaturases as they introduce double bonds into the fatty acyl chains between the Δ9 bond and the carboxyl end of the molecule, in contrast to the Δ12/ω6 and ω3 desaturases that produce C_{18} PUFA, which introduce double bonds between the Δ9 double bond and the methyl end of the chain [62]. In eukaryotic algae, Δ8, Δ6, Δ5 and Δ4 desaturases necessary for the production of EPA and DHA from 18:3n-3 have all been described [18] (Fig. 4).

The biosynthesis of n-3 LC-PUFA in marine bacteria also occurs through the activity of a bacterial PKS-type multi-enzyme complex [63]. The PKS pathway for LC-PUFA biosynthesis operates in some thraustochytrids, including *Schizochytrium* sp. [30], and is speculated to also possibly be responsible for DHA production in some flagellate microalgae [13]. In bacteria, the EPA biosynthesis gene cluster carries around 18 open reading frames (ORF), but only five of the genes termed pfaA, pfaB, pfaC pfaD and pfaE code for proteins required for the biosynthesis of EPA [63]. Analysis of the amino acid sequences derived from the genes showed that they were related to microbial PKS (and FAS) complexes [28]. Organization of the gene clusters is divided into three types with Type I, present in *Shewanella pneumatophori* SCRC-2738, containing all five pfa genes in close vicinity. Type II, as in *Moriella marina* MP-1, consists of a cluster of four genes pfaABCD with a separate pfaE, and Type III is considered to consist of just four genes with pfaE integrated into pfaC/E [27].

Biosynthetic enzymes including front-end desaturases and PUFA elongases activities have been also reported in fungi. The filamentous fungus *Mortierella alpina* has an elongase (GLELO) with the ability to elongate 18:3n-6 to 20:3n-6 and thus involved in ARA biosynthesis [21]. Additionally, desaturases with Δ5 [64] and Δ6 [65, 66] activities have been also identified. Other fungi from which desaturase genes have been identified include *Rhizopus nigricans*, *R. arrhizus*, *Mucor rouxii* and *Pythium irregulare* [67].

3. Fatty acyl desaturases and their genes in Bilateria

Other than the examples of the nematode *C. elegans* and some insects (Section 2.3.2), bilaterian organisms appear to lack enzymes capable of producing C_{18} PUFA from monounsaturated fatty acids [39]. Therefore, the production of LC-PUFA in most animals, and certainly in all vertebrates, is restricted to biosynthesis from pre-existing (dietary) C_{18} PUFA. The pathway for the production of LC-PUFA from C_{18} PUFA using aerobic front-end fatty acid desaturases (Fads) and fatty acid elongases (Elovl) is shown in Figure 4. The PUFA substrates for the pathway are 18:3n-3 and 18:2n-6 with DHA and 22:5n-6, respectively, generally being the most highly unsaturated products [68]. All the enzymes involved act on both the n-3 and n-6 series fatty acids with generally a preference for n-3 [38]. Using the n-3 example, production of EPA requires Δ6 and Δ5 desaturases producing 18:4n-3 from ALA and EPA from 20:4n-3, respectively. An alternative pathway for the production of 20:5n-3 operates via elongation of ALA to 20:3n-3 followed by Δ8- and Δ5-desaturation steps [69, 70]. In some basal vertebrate lineages, such as teleosts, the production of DHA from EPA can occur directly via a Δ4 desaturase that produces DHA from the EPA elongation product, 22:5n-3 [71]. In mammals, the production of DHA from EPA appears to involve a more complicated pathway that involves sequential elongations of EPA to produce 24:5n-3 that is desaturated by Δ6 desaturase [72] (Fig. 4). Recently, the human FADS2 gene product was demonstrated to have the ability for direct Δ4 desaturation of 22:5n-3 to 22:6n-3 [73].

Bilateria including invertebrate and vertebrate organisms possess genes encoding desaturases and elongases involved in biosynthetic pathways of LC-PUFA, although the exact repertoire and function remain to be clarified. In particular, this section will largely focus on front-end desaturases (Fads) with Δ4, Δ5, Δ6 and Δ8 activities, and Elovl2-, Elovl4- and Elovl5-like elongases found in chordates. Thus, the current knowledge on gene complement and functionalities of Fads and Elovl involved in LC-PUFA biosynthesis will be revised from basal vertebrates like cartilaginous fish to mammals. For the purpose of providing the adequate evolutionary perspective, the Fads and Elovl genes of non-chordate invertebrates will be also described.

3.1 Fads and Elovl in invertebrates and non-vertebrate chordates

Some invertebrate groups, and particular species among them, are becoming increasingly popular model species for comparative genomics and evolutionary developmental biology.

This is particularly true for species that have been noted for the retention of ancestral features of their genome organization as well as gene content to a much greater degree than seen in more traditional invertebrate model species [74-86]. As a result, ever-increasing genomic data of certain species are becoming available that will surely allow the study of genes encoding PUFA biosynthetic enzymes. While an extensive review of the molecular mechanisms of PUFA biosynthesis in marine invertebrates has been published recently [77], it is still relevant for the present review to highlight the most important findings uncovered in non-vertebrate metazoans, to contextualise the gene complement of Fads and Elovl in chordates, the focus of this review.

The nematode *C. elegans* was the first animal from which front-end desaturases, including Δ6 [41] and Δ5 [78, 79] desaturases, were isolated and characterized. More recently, further molecular studies on Fads and Elovl genes have been performed on another group of invertebrates, the molluscs. A complementary DNA (cDNA) encoding a Fads enzyme was first isolated from the common octopus *Octopus vulgaris* and shown to have Δ5 desaturation activity towards saturated and polyunsaturated fatty acyl substrates [80]. Likewise, a full-length cDNA sequence encoding an Elovl-like protein identified from *O. vulgaris* had high homology to vertebrate Elovl2 and Elovl5 enzymes [81]. Consistent with vertebrate Elovl2 and Elovl5 that have demonstrated roles in PUFA biosynthesis in vertebrates [20], the octopus Elovl was also efficient in the elongation of C18 and C20 PUFA substrates. Further investigations on other mollusc class representatives including *Haliotis discus hannai* (Gastropoda) [82] and *Chlamys nobilis* (Bivalvia) [83, 86], have confirmed the presence of Fads and Elovl with similar substrate specificities to those described previously for the *O. vulgaris* genes. More recently, two Fads- and Elovl-like cDNAs have been isolated and functionally characterized from the common cuttlefish *Sepia officinalis* [85]. These results confirm that the Fads and Elovl repertoire in protostome metazoans appear to have conserved functionalities.

Through the interrogation of transcriptome and genome sequences, Surm and co-workers [86] have recently screened a representative list of Fads and Elovl genes in a number of commercially important species within phylum Mollusca. It was confirmed that orthologues of Fads and Elovl were found in all investigated species covering three major classes including gastropods, bivalves and cephalopods. Regarding the Fads complement, cephalopods were found to possess one single Fads gene as previously predicted [80, 85], whereas a lineage-specific gene duplication event accounted for the presence of more than one Fads copy in some of the gastropod and bivalve species investigated. Interestingly, one

group of sequences (Clade A) included all the mollusc Fads that have been functionally characterized to date as Δ5 desaturases, but none of the Fads-like enzymes from Clade B have yet been functionally characterized [86]. Similar to Fads, gene duplication has played a major role in the evolution of the Elovl gene family in molluscs. Genomic data support the idea that a duplication event in the Elovl gene family likely occurred during the early diversification of bilateria as all three classes considered (cephalopods, bivalves and gastropods) possess sequences orthologous to the Elovl2/5- and Elovl4-like types found in present day vertebrates [86].

The characterization of *elovl*/*fads* gene repertoire and function in invertebrate deuterostomes including species from phyla such as the hemichordates, echinoderms, cephalochordates and tunicates is poorly detailed (Fig. 5). Here we focus on basal invertebrate chordate lineages. To date, a single *elovl* gene representative has been characterized in the European amphioxus (*Branchiostoma lanceolatum*) [87] and the sea squirt (*Ciona intestinalis*) [88]. Despite their clear orthology towards the larger super-family of ELOVL enzymes, phylogenetic analysis indicated that these characterized sequences belonged to different Elovl classes. Independent phylogenetic reconstructions demonstrated that the isolated sequence from the sea squirt belonged to the Elovl4 clade [87, 88], while the amphioxus gene was a *bona-fide* orthologue of Elovl2/5 from vertebrates [87]. Perhaps more interesting was the fact that close inspection of the available genomes of the Florida amphioxus (*Branchiostoma floridae*) and two sea squirt species (*C. intestinalis* and *C. savignyi*) suggested a fundamental difference. While orthologues of *elovl4* and *elovl2/5* were recognizable in the lancelet (amphioxus) genome (as they are indeed in protostomes), no apparent *elovl2/5* was found in these tunicate species [87] (Fig. 5). Some data on the characterization of the substrate specificities of these Elovl are also available. The Elovl4 from the sea squirt was able to elongate C_{18} and C_{20} PUFA [88]. In contrast, the amphioxus Elovl2/5 showed a more broad elongation pattern with the ability to elongate C_{22} PUFA, but with clear preferences for C_{18} and C_{20} [87]. At present no characterization of the orthologue of Elovl4 has been performed in amphioxus. Regarding desaturases, both amphioxus and sea squirts have a single desaturase gene although their precise phylogenetic relationships and functions have not yet been addressed (Fig. 5) [87, 89]. Preliminary analysis suggests that the tunicate desaturase is not a Fads but rather a sphingolipid desaturase (Castro and Monroig, personal communication).

3.2 Fads and Elovl in vertebrate basal lineages

3.2.1 Agnathans, chondrichthyans and holosteans

The Elovl and Fads gene repertoire in basal vertebrate lineages, such as chondrictyans and especially agnathans, is poorly investigated. Therefore, only patchy knowledge of the functions and diversity of LC-PUFA biosynthetic enzymes outside tetrapods, such as birds and mammals, and teleosts is currently available [90-92]. However, recent findings have provided some clarification of Fads and Elovl genes in early vertebrate evolution, and their overall impact on our understanding of the evolution of the LC-PUFA pathway. The gene complement of *fads* desaturases in chondrictyans was shown to partially coincide with that of mammals [89]. Thus, orthologues of both *fads1* and *fads2* were identified in the elasmobranch, the lesser-spotted dogfish or small-spotted catshark (*Scyliorhinus canicula*), pushing back the origin of these desaturase gene lineages to the pre-gnathostome period [89]. Moreover, the overall PUFA substrate specificity of both gene orthologues showed unequivocally that Fads1 and Fads2 were Δ5 and Δ6 desaturases, respectively, similar to that previously determined in mammals [89]. The recent release of the genome sequence of the holocephali species, the elephant shark (*Callorhinchus milii*) [93], confirmed the initial description in the dogfish, with only two Fads-encoding genes found in the chimaera genome (Fig. 6). *Fads* genes tend to occur in genomes in physical clusters, an indication of their origin through tandem gene duplication. That is the case in humans with a gene cluster with three Fads genes, as well as birds and reptiles with four and five Fads genes, respectively [89]. Although the original description of the *fads* repertoire in cartilaginous fish did not provide information regarding the genomic organization of these genes, the *fads1* and *fads2* orthologues in *C. milii* map together into the same genomic scaffold with a similar composition to the human *FADS* gene *locus* at chromosome 11 (Fig. 6). The finding of *fads1* and *fads2* orthologues in cartilaginous fish, together with the description of a single basal gene in invertebrate chordates [89], provided tentative evidence that both genes originate from a gene duplication dated back to the transition from cephalochordates/tunicates to gnathostomes. In the most basal vertebrate branch, the agnathans, the repertoire of Fads genes is presently unknown (Fig. 5). While the genome of the sea lamprey (*Petromyzon marinus*) presented no detectable gene sequence with similarity to front-end desaturases, the existence of a partial sequence with similarity to *fads2* was suggested previously [89]. Moreover, the recently available genome from the Japanese

lamprey (*Lecentheron japonicus*) [8] indicated the presence of at least a single sequence with similarity to *fads1* (Castro's personal communication) (Fig. 5) and thus the currently available data enable us to tentatively infer the presence of *fads1* and *fads2* orthologues in agnathans. This would tentatively date the origin of these two gene lineages to the time of the invertebrate/vertebrate transition.

The *elovl* gene repertoire involved in LC-PUFA biosynthesis, namely Elovl2 and Elovl5, has been characterized in agnathans and chondrictyans. Monroig and co-workers [87] have investigated the *elovl2* and *elovl5* gene repertoire and function in two species belonging to these chordate lineages, the sea lamprey and the elephant shark (Fig. 5). In the case of the elephant shark, orthologues of the tetrapod Elovl2 and Elovl5 were identified and their origin delineated using both phylogenetics and comparative genomics [87]. However, agnathan sequences are often difficult to place phylogenetically [94]. In the case of Elovl2/5, supporting evidence from both phylogenetic and synteny comparisons indicated that orthologues of both Elovl2 and Elovl5 can indeed be found in lampreys [87] (Fig. 5). Analysis of the PUFA substrate specificities of these enzymes revealed a complex evolutionary pattern. The elephant shark Elovl resembled other gnathostome Elovl2 and Elovl5 with clear preferences for C_{22} and C_{18}, respectively, while a clear overlap was observed for C_{20} PUFA substrates [87]. The profile determined for the lamprey genes was strikingly different. The agnathan putative Elovl2 and Elovl5 enzymes showed overlapping preferences for C_{18} and C_{20}, respectively, with no capacity to elongate C_{22} PUFA [87]. These results suggested that the agnathans represent an intermediate state after duplication since they completely lack the ability to elongate C_{22} PUFA observed in cartilaginous fish such as elephant shark.

The exact role and gene number of *elovl4* genes in lampreys and cartilaginous fish has not been described or characterized previously. However, genome mining clearly indicated the presence of single copy orthologues in both lineages, which await functional characterization (Fig. 5). Finally, other emerging genomic fish models will most likely provide a better-defined picture of Fads and Elovl evolution. For example, the impact of the teleost-specific whole genome duplication (3R) has been barely explored considering other non-classical vertebrate models such as the spotted gar, *Lepisosteus oculatus*, a pre-3R holostean [4]. Scrutiny of the spotted gar genome revealed the identification of the full gene complement of Fads (*fads1* and *fads2*) and Elovl (*elovl2*, *elovl4*, and *elovl5*), an indication that a conserved LC-PUFA pathway is probably present (Castro's personal communication).

546 *3.2.2 Teleosts*

547

548 The mechanisms of LC-PUFA biosynthesis in teleost fish, particularly farmed species,
549 have been investigated extensively in recent years, and many aspects of these metabolic
550 pathways are better understood in fish compared to mammals. These investigations have led
551 to the conclusion that LC-PUFA biosynthetic capability varied notably among species linked
552 to differences in the complement of Fads and Elovl gene and enzymatic activities existing in
553 that species [92]. Generally, freshwater and salmonid species have been regarded as having
554 high capacity for LC-PUFA biosynthesis, whereas marine teleost species showed a limited
555 ability to biosynthesize LC-PUFA [95]. This has been hypothesized to be a consequence of
556 marine species having evolved in an LC-PUFA-rich environment and thus with low
557 evolutionary pressure to retain the ability to endogenously produce LC-PUFA. In contrast,
558 lower levels of LC-PUFA in the food chain may have been the selective pressure driving
559 freshwater species to retain the ability to biosynthesize LC-PUFA to satisfy their
560 physiological requirements [89, 95, 96]. In addition to the fact that such dichotomy had been
561 established based on a rather limited number of species, other confounding factors beyond
562 habitat have been explored in recent years. Among them, the trophic level, the position of an
563 organism within the food web, the "trophic ecology" and diadromy have been also suggested
564 as potential drivers modulating the range of desaturation and elongation capabilities in
565 teleosts and thus their ability for LC-PUFA biosynthesis [97, 98]. Importantly for the purpose
566 of this review, it has become obvious that the capability for LC-PUFA biosynthesis in teleost
567 fish is more diverse than in other vertebrate groups, and is possibly the result of a
568 combination of factors that interact throughout the evolutionary history of each particular
569 group or species.

570

571 3.2.2.1 Fads in teleosts

572

573 Teleosts have been by far the group of tetrapods where the greatest number of Fads and
574 Elovl have been studied (Fig. 5). The first Fads-encoding cDNAs were isolated from rainbow
575 trout [99] and zebrafish [100]. The amino acid sequence homology of the two Fads indicated
576 they both were Δ6 desaturases. However, the functional characterization of the zebrafish Fads
577 by heterologous expression in yeast demonstrated that the encoded enzyme was a bifunctional
578 Δ6/Δ5 desaturase [100]. Subsequently, a relatively large number of Fads-like cDNAs have
579 been isolated from a variety of teleost fish from a wide range of environments (from

freshwater species such as zebrafish, to diadromous species like Atlantic salmon, and marine species such as Atlantic cod), trophic levels (from true herbivores like rabbitfish to top carnivores like Atlantic bluefin tuna) and phylogenetic groups (from the basal teleost clade Elopomorpha such as eels, to Percomorpharia like cobia or gilthead seabream). An extensive list of all Fads-like cDNAs that have been isolated and functionally characterized from teleost fish species is shown in Table 1 [70, 71, 92, 97-117].

It has been shown that all the Fads-like desaturases characterized in teleost fish are *FADS2* orthologues [89]. Interesting, whereas mammalian *FADS2* are predominantly Δ6 desaturases, teleostei Fads2 enzymes show much more varied substrate specificities (Table 1). Similar to mammalian orthologues, many fish Fads2 are indeed typical Δ6 desaturases as demonstrated in a range of marine (gilthead sea bream, turbot, Atlantic cod, cobia, European sea bass, barramundi, Northern bluefin tuna, meagre, Nibe croaker) and freshwater species (common carp and rainbow trout). None of those Fads2 showed Δ5 or Δ4 desaturase activities. In Atlantic salmon, however, four distinct genes encoding Fads2 have arisen as a result of segmental duplications and not as a result of the salmonid tetraploidization event hypothesized to have occurred between 25 and 100 million years ago [104]. While the so-called Δ6Fad_a, Δ6Fad_b, and Δ6Fad_c [104] were shown to have Δ6 specificity [103, 104], a fourth Fads2 cDNA was demonstrated to have Δ5-desaturase activity [102]. The salmon Δ5 Fads2, together with the three Δ6-like desaturases described above, enable this species to perform all desaturation reactions (i.e. Δ6 and Δ5 desaturations) required in the LC-PUFA biosynthesis through the conventional pathways for mammals (Fig. 4). Thus, biosynthesis of DHA in Atlantic salmon appears to proceed through the Sprecher pathway. This is also the case for rainbow trout, for which another monofunctional Δ5 Fads2 has been characterized (Hamid's personal communication), and zebrafish, the only fish species where there is supporting evidence for the existence of the Sprecher pathway in fish [118-120]. Interestingly, other teleost Fads2 have apparently lost the ability to desaturate 24:5n-3 to 24:6n-3, a key step in the Sprecher pathway. Thus, the Nibe croaker *Nibea mitsukurii*, a member of the Sciaenidae family (Percomorpharia) [121], possess a Fads2 that has Δ6 desaturase activity towards 18:3n-3 but no capability to desaturate 24:5n-3 [112]. Whether the apparent loss of Δ6 desaturase activity on C_{24} substrates in Fads2 of *N. mitsukurii* is an extended trait or not among fish Fads2 desaturases remains to be elucidated. However, it has become apparent in recent years that Fads2 from teleosts have evolved quite markedly from the typical Fads2 functionalities as described in mammals as indicated by their high plasticity in terms of substrate specificities. Other than the abovementioned zebrafish bifunctional Δ6Δ5 Fads, and

614 the salmon and trout Δ5 Fads2, other subfunctionalized Fads2 have been subsequently
615 characterized in several fish species.
616 Arguably the most important and interesting finding in our investigations of teleost LC-
617 PUFA biosynthesis in recent years has been the discovery of a Fads2 in rabbitfish (*Siganus*
618 *canaliculatus*) with capability for Δ4 desaturation [71], an activity originally believed to
619 account for the production of DHA directly from 22:5n-3 resulting from a single elongation
620 of EPA [68]. More recently Fads2 with Δ4 activities have also been characterized in
621 Senegalese sole *Solea senegalensis* [97], pike silverside *Chirostoma estor* [92] and the striped
622 snakehead *Channa striata* [117]. Further to the isolation and characterization of Δ4 desaturase
623 cDNAs in teleosts, *in vivo* biochemical evidence of the existence of an enzymatic activity
624 enabling Δ4-desaturase-dependent DHA synthesis has been shown recently in *S. senegalensis*
625 [122]. In comparison with the Sprecher pathway, the so-called "Δ4 pathway" is a more direct
626 metabolic route as it avoids translocation of LC-PUFA intermediates (namely 24:6n-3)
627 between endoplasmic reticulum and peroxisomes, and also the further catabolic step (partial
628 oxidation to DHA) occurring in the latter organelle [72, 123]. In addition to the Δ4 Fads2,
629 rabbitfish also expressed a bifunctional Δ6Δ5 Fads2 [71], similar to the zebrafish desaturase,
630 and the high degree of amino acid (aa) sequence similarity between the two rabbitfish Fads2
631 has prompted investigation of the specific residues dictating the different substrate
632 specificities of Fads2 desaturases [124]. It was concluded that four aa residues (YNYN for Δ4
633 Fads2 and FHYQ for Δ6Δ5 Fads2) located between the second and third histidine boxes were
634 responsible for the PUFA substrate specificities of each enzyme. *In silico* searches among
635 teleost fish indicated that Fads2 with the key aa residues and thus being putative Δ4
636 desaturases were more widely spread than initially believed. For instance, cichlids including
637 *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni* and *Pundamilia nyererei*, all
638 possess putative Δ4 Fads2 (Monroig's personal communication).
639 As mentioned above, plasticity of teleost Fads2 is not restricted to the existence of
640 monofunctional Δ6, Δ5 or Δ4 desaturases, but some of the enzymes investigated have been
641 shown to be bifunctional proteins. In addition to the abovementioned zebrafish Fads2 that was
642 the first bifunctional Δ6Δ5 desaturase described [100], other bifunctional desaturases were
643 described in fungi [54, 125], protozoans [53] and moths [126]. In fish, the rabbitfish *S.*
644 *canaliculatus* [71] as described above, Nile tilapia *O. niloticus* and pike silverside *C. estor*
645 [92] also possess bifunctional Δ6Δ5 Fads2 enzymes. These bifunctional desaturases are
646 enzymes that can introduce two separate, distinct double bonds into the same fatty acid chain
647 and, interestingly, mammalian Fads2, zebrafish Δ6Δ5 Fads2 and possibly salmon Fads2_a,

are also believed to be responsible for introducing two distinct bonds into both C_{18} and C_{24} chains as required for DHA synthesis [120, 127, 128]. Many Fads2 desaturases are also able to introduce the same double bond into both C_{18} and C_{20} chains. Specifically, a retrospective study investigating the ability of Fads2 from freshwater, diadromous and marine teleost species to desaturate PUFA at position Δ8, an intrinsic characteristic of mammalian FADS2 [69], concluded that is also a characteristic of teleost Fads2. That was also the case of bifunctional Δ6Δ5 desaturases, and so this sort of multifunctionality appears to be an extended feature among teleost Fads2. Furthermore, the Δ8 activity varied notably among the species investigated, with marine fish Fads2 having higher Δ8 capability than Fads2 from freshwater/diadromous species. The establishment of Δ8 activity in Fads2 enzymes opened up the possibility of a "Δ8 pathway" whereby 20:3n-3 was no longer regarded as a dead-end product but could potentially be an intermediate in the LC-PUFA biosynthetic pathway. However, it remained unclear what benefit this evolutionary adaptation implied for marine species that are receiving adequate dietary EPA and DHA, and little 18:3n-3 or 20:3n-3, and in which the limiting step in the LC-PUFA biosynthesis pathway appears to be lack of Δ5-desaturation capability [13, 129]. It is important to note that, while the desaturase gene numbers and substrate specificities vary among species, some teleosts appear to lack any *fads*-like gene in their genomes. This is the case of two pufferfish species, namely *Takifugu rubripes* and *Tetraodon nigroviridis* [129].

3.2.2.2 Elovl in Teleosts

Elovl-like cDNAs have been isolated and characterized from a wide range of fish species (Table 2) [92, 96-98, 107, 110-113, 115, 130-140]. The first teleost Elovl-like cDNAs with a role in LC-PUFA biosynthesis was a zebrafish elongase that was identified as an Elovl5 enzyme [130]. Similar to mammalian orthologues [141, 142], zebrafish Elovl5 showed the ability to elongate C_{18} and C_{20} PUFA, with markedly lower elongation efficiency on C_{22} PUFA substrates. Subsequently, Elovl5 cDNAs were cloned and characterized in Atlantic salmon, the only teleost species where two paralogues have been cloned and functionally characterized [102, 134], catfish, tilapia, turbot, gilthead sea bream, Atlantic cod [133], cobia [107], barramundi [110], Southern [137] and Northern [113] bluefin tuna, rabbitfish [138], black seabream [139], meagre [98], rainbow trout [140] and Nibe croaker [112]. While the majority of these studies on the functional characterization of Elovl5 included only 18:4n-3 and 18:3n-6 as substrates for C_{18} elongation, the more recent studies increasingly include

both 18:3n-3 and 18:2n-6 as potential substrates for elongation [98, 137]. Consequently, Elovl5 has been demonstrated to have the ability to elongate 18:3n-3 and 18:2n-6 and thus produce adequate Δ8-desaturation substrates 20:3n-3 and 20:2n-6 (Fig. 4). The establishment of the ability of Elovl5 to elongate 18:3n-3 and 18:2n-6 along with the aforementioned Δ8 activity in Fads2 enzymes has confirmed the possibility of a "Δ8 pathway" and so the initial steps in LC-PUFA biosynthesis can be performed by the same Fads2 and Elovl5 enzymes but with either activity initiating the pathway, desaturase followed by the elongase (Δ6) or elongase followed by desaturase (Δ8). Interesting, the meagre *A. regius* and the Nibe croaker *N. mitsukurii* Elovl5 have also shown the ability to elongate the C_{16} PUFA 16:3n-3 [98, 112], a fatty acid found in algae and plants. Moreover, heterologous expression in yeast suggested a putative role for Elovl5 in fish in the elongation of monounsaturated fatty acids, as denoted by the endogenous 18:1n-7 and 18:1n-9 in yeast being elongated to 20:1n-9 and 20:1n-7 [98, 102, 110, 112, 134]. The teleost Elovl5 functionalities suggest that these are more versatile and adaptive enzymes than the mammalian orthologues. In this regard, adaptation to diet was postulated as the main evolutionary driver accounting for the different tissue expression of the two Elovl5 paralogues found in Atlantic salmon, and that of Northern pike (*Esox lucius*), the closest extant relative to the preduplicated ancestral salmonid [96]. Thus, the complementary expression pattern of *elovl5* genes in Atlantic salmon (liver and intestine) might have enabled Atlantic salmon to thrive in a relatively poor LC-PUFA environment during the freshwater stages. It was postulated that Atlantic salmon *elovl5* genes have been efficiently retained in the genome under strong functional constraints indicating a physiological requirement for both enzymes to be functionally active. On the other hand, a piscivorous species like the Northern pike, despite it has the ability to biosynthesize DHA from ALA [143], can directly satisfy its LC-PUFA requirements in the diet and thus the Elovl5 activity for LC-PUFA production in liver and intestine might not be so critical. Upstream promoter regions of salmon *elovl5* have strongly diverged from one another, and thus, in contrast to their similar substrate preferences, the different regulatory mechanisms indicate a relaxation of purifying selection following the duplication event [144].

Investigations of the other Elovl enzymes involved in LC-PUFA biosynthesis, i.e. Elovl2 and Elovl4, has contributed considerably to an advanced understanding of these metabolic pathways in teleosts (Fig. 5). An Elovl2 enzyme was cloned and functionally characterized for the first time in Atlantic salmon [134] and soon thereafter in zebrafish [131]. While showing some low level of elongation activity towards C_{18} substrates (18:4n-3 and 18:3n-6), the Elovl2 were particularly efficient in the elongation of C_{20} (20:5n-3 and 20:4n-6) and C_{22}

716 (20:5n-3 and 20:4n-6) LC-PUFA substrates, the latter not regarded as substrates for Elovl5
717 according to the low (if any) conversions observed in the yeast expression system. Recently,
718 an Elovl2 has been isolated and investigated in rainbow trout *O. mykiss* [140]. Similar to the
719 observations in *S. salar* and *D. rerio*, the *O. mykiss* Elovl2 showed the ability to elongate C_{20}
720 and C_{22} but, in contrast, did not show any activity on C_{18} PUFA substrates. Interestingly it is
721 unclear whether the ability to elongate C_{18} PUFA is a common feature among Elovl2 proteins
722 as, like Atlantic salmon and zebrafish orthologes, the mouse ELOVL2 showed some
723 elongation capability on C_{18} PUFA [141], although the human and chicken did not (see
724 below), similar to rainbow trout Elovl2 [90, 142]. Regardless of its possible role in C_{18} PUFA
725 elongation, the ability of Elovl2 to elongate 22:5n-3 to 24:5n-3 was regarded as key for the
726 production of DHA via the "Sprecher pathway", and because of the loss of Elovl2 in the vast
727 majority of marine fish lineages and virtually all commercially important farmed species, this
728 has been hypothesized as a possible factor accounting for the limited ability for DHA
729 biosynthesis in those species [134].
730 Elovl4 are the most recent member of the Elovl family that has been studied in teleosts.
731 Zebrafish was the first teleost fish species from which Elovl4 were isolated and functionally
732 characterized, representing at that time the only report of a non-human Elovl4 elongase [132].
733 Two isoforms, termed as Elovl4a and Elovl4b, were described in zebrafish and both showed
734 the ability to efficiently elongate saturated fatty acids up to C_{36}. Interestingly though, activity
735 for the elongation of PUFA substrates was only shown by Elovl4b, which effectively
736 converted the C_{20} LC-PUFA, EPA and ARA, to elongated products with acyl chain-lengths
737 up to C_{36}. Subsequent studies describing the cloning and functional characterisation of Elovl4
738 were conducted in cobia (*R. canadum*), Atlantic salmon (*S. salar*) and rabbitfish (*S.*
739 *canaliculatus*) [135, 136, 138]. In agreement with these enzymes being homologues of the
740 zebrafish Elovl4b isoform, the cobia, Atlantic salmon and rabbitfish Elovl4 enzymes showed
741 the ability to biosynthesize both saturated and polyunsaturated VLC-FA. While Atlantic
742 salmon also possess an Elovl2 with elongation capability towards C_{22} LC-PUFA [134], the
743 ability of Elovl4 from cobia and rabbitfish, two marine Actinopterygii species, to elongate
744 22:5n-3 to 24:5n-3 provided evidence to support a role for Elovl4 of some teleosts at least in
745 DHA biosynthesis similar to Elovl2 [136]. More recently, the Nibe croaker Elovl4 [112],
746 another Elovl4b-like orthologue, was shown to be capable of elongating 22:5n-3 to 24:5n-3
747 although the reported conversions were notably lower (3.6 %) compared to previously
748 reported conversions for zebrafish isoform b (29.8 %), cobia (34.1 %), Atlantic salmon
749 (22.1 %) and rabbitfish (20.7 %). However, these studies on fish Elovl4 elongases have

revealed that Elovl4 from some marine species can elongate 22:5n-3 to 24:5n-3 and other C_{22} LC-PUFA, and this has been hypothesized as an adaptive strategy to compensate for the lack of Elovl2 in these species [136]. *In silico* searches strongly suggest that all teleosts appear to possess at least one copy of both *elovl4a* and *elovl4b*. It is uncertain which mechanism has led teleosts to have two distinct genes for Elovl4 enzymes, but it is clear that they may have diverged to acquire different functions and tissue distributions [132]. A comprehensive tissue distribution study on ten *elovl* genes performed in Atlantic cod (*Gadus morhua*) recently showed that, in addition to Elovl4a and Elovl4b homologues, this teleost possessed two further *elovl4*-like transcripts termed as *elovl4c-1* and *elovl4c-2* [145]. Further studies investigating the presence of this Elovl in other teleost species and its function are required.

3.3 Fads and Elovl in non-mammalian tetrapods

This section reviews the existing knowledge on LC-PUFA biosynthetic enzymes in reptiles, birds, and amphibians. Basal tetrapods such as amphibians display the complete repertoire of Fads and Elovl although their function has not been elucidated (Fig. 5). In birds and reptiles an interesting finding was described with respect to the Fads gene collection as investigation into the genomes of representative species showed a unique Fads1 gene expansion in this lineage (Fig. 5) [89]. It is unclear at this stage whether these enzymes perform different functions, for example utilizing alternative PUFA substrates (sub-functionalization), or if they have evolved novel functions with respect to PUFA desaturation. Since this gene expansion dates back to reptile/bird separation we anticipate that these genes will perform similar functions in both lineages. Additionally, given the relevance of poultry production it is important that future studies investigate Fads capacities in chicken.

The chicken (*Gallus gallus*) ELOVL2 and ELOVL5 were the first elongase genes to be studied in birds [90] (Table 3). Functional characterization of the two proteins in the heterologous yeast *S. cerevisiae* showed the ELOVL2 was capable of elongating C_{20}-C_{22} PUFA substrates, and no activity on C_{18} PUFA such as 18:4n-3 and 18:3n-6 being detected. In contrast, the functionality of the chicken ELOVL5 was reported to be broader compared to ELOVL2. Thus, the chicken ELOVL5 showed the ability to elongate a variety of substrates including C_{18} (18:4n-3 and 18:3n-6), C_{20} (EPA and ARA) and, interestingly, C_{22} (22:5n-3 and 22:4n-6) PUFA. Indeed, the ability of the chicken to produce 24:5n-3 from EPA was regarded as unique when compared to non-piscine orthologues such as human [142] and rat [146]. However, as discussed above (Section 3.2.2.2), Elovl5 from some teleost fish showed some

784 limited ability to elongate C_{22} PUFA including 22:5n-3 and, as suggested for the chicken
785 ELOVL5, they can potentially contribute to the biosynthesis of DHA through the Sprecher
786 pathway [72].
787 The ability of the chicken ELOVL5 to elongate C_{22} PUFA does not appear to be a common
788 feature among bird ELOVL5. Thus, a subsequent study on fatty acid elongases of the
789 domestic Mallard duck (*Anas platyrhynchos*) and Australian white hybrid turkey (*Meleagris*
790 *gallopavo*) showed that the Elovl5 from these species had no activity towards C_{22} PUFA
791 including 22:5n-3 and 22:4n-6 [91]. Unlike the chicken ELOVL5, both the duck and turkey
792 ELOVL5 had C_{18} PUFA such as 18:4n-3 and 18:3n-6, as well as C_{20} PUFA like EPA and
793 ARA as preferred substrates for elongation. Similarly, functional analyses of the duck and
794 turkey ELOVL2 also showed some differences compared to the chicken orthologue [90].
795 Although the turkey ELOVL2 did not show any activity towards C_{18} PUFA substrates like the
796 chicken ELOVL2, the turkey orthologue also showed no activity on ARA that remained
797 unmodified. The duck ELOVL2 was capable of elongating C_{20} (including EPA and ARA) and
798 C_{22} substrates similar to the chicken ELOVL2 but, in addition, it showed the ability to
799 elongate C_{18} PUFA like 18:4n-3 and 18:3n-6, an capability not shown by the chicken
800 orthologue. The ability of Elovl2 proteins to elongate C_{18} PUFA substrates to a small extent
801 has also been described in fish as detailed above [131, 134] and mouse [142].
802
803 3.4. FADS and ELOVL in mammals
804
805 The LC-PUFA biosynthetic enzymes have been extensively studied in human and other
806 mammals, primarily rodent model species such as mouse *Mus musculus* and rat *Rattus*
807 *norvegicus*. As a result, several reviews have been published in recent years [20, 22, 32].
808 Therefore, this section will provide a brief overview of the *FADS* and *ELOVL* gene
809 complement in mammals and the functions of their protein products with a role in LC-PUFA
810 biosynthesis. Additionally, we will also discuss the most recent advances on evolutionary
811 aspects of these gene families.
812
813 *3.4.1 Mammalian FADS*
814
815 Mammals express Δ6 and Δ5 desaturases with the ability to desaturate at the Δ6 and Δ5
816 positions of PUFA substrates. In humans the Δ5 and Δ6 desaturase enzymes are encoded by
817 the *FADS1* and *FADS2* genes, respectively [147] (Fig. 5). These genes are organized into a

818 tight physical cluster on chromosome 11, and a further desaturase, FADS3 with unknown
819 function [148], is also part of the cluster [147, 148]. Interestingly, Castro and co-workers [89]
820 also identified a fourth Fads gene (*Fads4*) sequence occurring at a distant genomic location of
821 the *FADS1/FADS2/FADS3* gene cluster in mouse. The function and role of murine *Fads4*
822 remains to be characterized, but its unique phylogenetic distribution and rather restricted
823 occurrence in some mammalian species, perhaps suggests a very specific role.
824 By comparison of the sequences of known Δ6 desaturases from a cyanobacterium [150],
825 nematode [41] and plant [151], full lengths cDNAs for Δ6 FADS2 desaturases were
826 characterized in mammals including rat [152], mouse and human [153] (Table 3). Functional
827 characterization demonstrated that the C_{18} PUFA, 18:3n-3 and 18:2n-6, were adequate
828 substrates for the mammalian Δ6 desaturases and, subsequently, it was demonstrated that the
829 Δ6 desaturase from human [127] and rat [128] also had the ability to desaturate 24:5n-3 at the
830 Δ6 position [127]. While the recent discovery of Δ4 activity of the human FADS2 provides an
831 alternative and direct pathway for DHA biosynthesis [73], historically it was accepted that
832 DHA production was achieved through the Sprecher pathway and, thus, FADS2 mediates the
833 Δ6 desaturation of 24:5n-3 to 24:6n-3, which is thereafter translocated to peroxisomes for
834 chain shortening via partial β-oxidation [123]. This makes DHA biosynthesis by the Sprecher
835 pathway a somewhat inefficient process and thus Δ6 desaturase has been regarded as the
836 overall rate-limiting factor in the LC-PUFA biosynthetic pathway in mammals [22]. Unlike
837 Δ6 desaturases, the Δ5 desaturases operate at one single step of the pathway, converting
838 20:4n-3 to EPA in the n-3 series, and 20:3n-6 to ARA in the n-6 series (Fig. 4). Several
839 mammalian Δ5 FADS1 desaturase genes have been characterized from human [154], rat
840 [155] and mouse [156]. Interestingly, non-coding RNA genes, which are transcribed from the
841 opposite strand of the mammalian *FADS1* gene, were later discovered in human, mouse and
842 rat [157]. Such reverse Δ5-desaturase was found to regulate the expression and enzymatic
843 activity of the rat FADS1, supporting a significant role as a natural antisense regulator of Δ5-
844 desaturase.
845 In addition to the above described Δ6 desaturation capability of mammalian FADS2, this
846 enzyme can also operate as a Δ8 desaturase and thus PUFA substrates including 20:3n-3 and
847 20:2n-6 can be desaturated to 20:4n-3 and 20:3n-6, respectively, to thus reincorporate them
848 into the pathway for further Δ5 desaturation [69] (Fig. 4). It is worth mentioning that the Δ8
849 desaturase capacity of the baboon FADS2 [69] seemed to be notably lower compared to those
850 of marine teleost Fads2 assayed under similar conditions [70]. These findings represent
851 further evidence of subfunctionalization occurring in teleost Fads2. Importantly, while the

852 teleostei Elovl5 has been identified to elongate C_{18} PUFA (18:3n-3 and 18:2n-6) required for
853 the Δ8 pathway, it still remains to be elucidated if the mammalian ELOVL5 is responsible for
854 such conversions [22]
855 Adaptive evolution studies have recently revealed that ability to biosynthesize LC-PUFA
856 varies notably among human populations and, for instance, levels of LC-PUFA in African-
857 American individuals were found to be higher compared to European-Americans [158, 159].
858 Such differences were linked to polymorphisms in the FADS gene cluster that then lead to
859 higher capacity to biosynthesis LC-PUFA from C_{18} precursors. It was postulated that highly
860 efficient desaturation alleles of *FADS* genes are particularly abundant in African populations
861 where these were retained by positive selection driven by dietary limitation of preformed LC-
862 PUFA in isolated regions [160].
863
864 *3.4.1 Mammalian ELOVL*
865
866 Seven members of the ELOVL protein family (ELOVL 1-7) have been described in
867 mammals [20, 22]. The elongation of saturated and monounsaturated fatty acids in mammals
868 can be catalyzed by four elongases, namely ELOVL1, ELOVL3, ELOVL6 and ELOVL7, that
869 show different tissue distributions probably reflecting different functional roles. ELOVL1 is
870 thought to be involved in the production of saturated fatty acids up to C_{26} in length, as present
871 in sphingolipids [161, 162]. It is expressed in most tissues, which may reflect a housekeeping
872 role and a requirement for these fatty acids and lipids containing them in membranes, but it is
873 also highly expressed in some parts of the central nervous system and may have an important
874 role in the synthesis of sphingomyelin and hence myelin [162, 163]. ELOVL3 is suggested to
875 control the synthesis of saturated and monounsaturated fatty acids of up to C_{24} and is
876 expressed mainly in brown and white adipose tissue, skin and liver [20, 164]. ELOVL3 is
877 cold inducible in brown adipose tissue [164], displays a diurnal expression in the liver, and is
878 regulated by gender-specific steroid hormones such as glucocorticoids, androgens and
879 oestrogens [165]. ELOVL6 is involved in the elongation of $C_{12\text{-}16}$ saturated fatty acids up to
880 C_{18}, but it has no capability to elongate beyond C_{18}. *ELOVL6* is ubiquitously expressed but
881 especially in liver, brain, and other tissues with high lipid content such as white and brown
882 adipose tissue [166, 167]. ELOVL7, the latest member of the ELOVL family to be identified,
883 is highly expressed in the kidney, pancreas, adrenal glands and prostate [168]. Knockdown
884 experiments of Elovl7 in carcinoma cell lines showed reduced levels of saturates 20:0, 22:0

and 24:0. Moreover, overexpression of Elovl7 in microsomes has confirmed that Elovl7 participates in elongation of saturated fatty acids with up to 24 carbons.

As with saturated and monounsaturated fatty acids, and as previously described for fish above, there are multiple enzymes for the elongation of PUFA in mammals that differ in tissue distributions and fatty acid specificity likely reflecting different functional roles. The *ELOVL2* and *ELOVL5* enzymes appear to be fairly ubiquitously expressed in most tissues in mammals although there can be species differences with *Elovl5* in rats showing highest expression in lung and brain whereas human *ELOVL5* is particularly highly expressed in testis and adrenal gland that are characterized by relatively high levels of 22:5n-6 [141, 167] (Fig. 5). These two elongases have overlapping fatty acid substrate specificity with ELOVL5 being able to elongate both C_{18} and C_{20} PUFA, but with no activity towards C_{22}, whereas ELOVL2 is generally able to elongate C_{20} and C_{22} PUFA [142, 166]. However, while mouse ELOVL2 was able to elongate 18:3n–6 to some degree, human ELOVL2 was not, which suggested there may be some functional divergence between species as discussed above in teleosts (Section 3.2.2.2). Neither ELOVL2 nor ELOVL5 have activity towards saturated or monounsaturated fatty acids [142, 166], contrary to what was observed with some teleost Elovl5 when expressed in yeast. The yeast expression system was also used to investigate the substrate specificities, competitive substrate interactions and dose response curves of the rat ELOVL2 and ELOVL5 [146]. It was confirmed that the rat ELOVL2 was active towards C_{20} and C_{22} PUFA but interestingly, the reaction converting the C_{22} PUFA, 22:5n-3 to 24:5n-3 appeared to be saturated at substrate concentrations and thus ELOVL2 was postulated as an additional control point of LC-PUFA biosynthesis in addition to FADS2 mentioned above.

In contrast to ELOVL2 and ELOVL5, ELOVL4 has much more specific tissue distribution being highly expressed in retina and brain but also skin [169, 170]. A major difference of ELOVL4 compared to the other mammalian ELOVL is that it is capable of elongating both saturated fatty acids and PUFA [20]. Therefore, ELOVL4 is responsible for the biosynthesis of saturated VLC-FA (e.g. 26:0, 28:0 and 30:0) that are components of sphingolipids and ceramides and important in the skin [171, 172], and also for the biosynthesis of VLC-PUFA ($> C_{24}$) that are components of key phosphatidylcholine species in retina [173-175], brain [14, 176] and testis [177-180]. Although some of these studies revealed potential substrates for mammalian ELOVL4, the specific steps in which ELOVL4 was involved was only described in detail later when the human orthologue of ELOVL4 was characterized [181]. Overexpression of human ELOVL4 in rat neonatal cardiomyocytes and a human RPE cell line ARPE-19 showed a decreased level of 26:0 and concomitant increased levels of 28:0 and

30:0. Similar conclusions were obtained in genetically engineered mice lacking a functional ELOVL4 protein, which showed increased levels of C_{26} fatty acids and a depletion of C_{28} in lipids of the epidermal stratum corneum, which altered the skin barrier function and ultimately caused dehydration and perinatal death [171, 172, 182]. In addition to saturated VLC-FA biosynthesis, the human ELOVL4 was also demonstrated to mediate the production of C_{28}-C_{38} n-3 VLC-PUFA, compounds mainly present into phospholipid molecules in retina [183]. As far as the authors are aware, no other mammalian ELOVL4 has been functionally characterized.

4. Concluding remarks

The extraordinary number of full genome sequences currently available offers a unique opportunity to investigate the evolution of complex gene pathways, and their impact on animal physiology. One of those examples is the genetic cascade controlling LC-PUFA biosynthesis, namely Fads and Elovl enzymes. In this review, we extensively addressed aspects directly linked with the diversity and function of Elovl and Fads genes in key chordate species. The existing literature supports the fundamental conclusion that the wiring and elaboration of a complete and functional LC-PUFA cascade evolved in the vertebrate lineage. The available data support an evolutionary model where a combination of whole genome and tandem duplications expanded the repertoire of Elovl and Fads in vertebrate ancestry, thus contributing to the diversification of these molecular modules – Elovl2, Elovl4, Elovl5, Fads1, and Fads2 (Fig. 7).

In this context, some questions remain to be addressed. The characterization of FADS enzymes in the amphioxus and agnathans will be essential to provide a defined picture at the invertebrate/vertebrate transition. Additionally, the functional characterization of Fads and Elovl genes retrieved from the sequenced genome of the holostean spotted gar, a basal freshwater fish closely related to teleosts, should facilitate our understanding of LC-PUFA biosynthesis. Also, Elovl4 orthologues remain largely uncharacterized as well their exact role in different species. Finally, the integration with functional *in vivo* studies to determine the interplay between endogenous synthesis versus dietary inputs of LC-PUFA is of vital importance to understand the impact and regulation at the physiological level.

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Figures

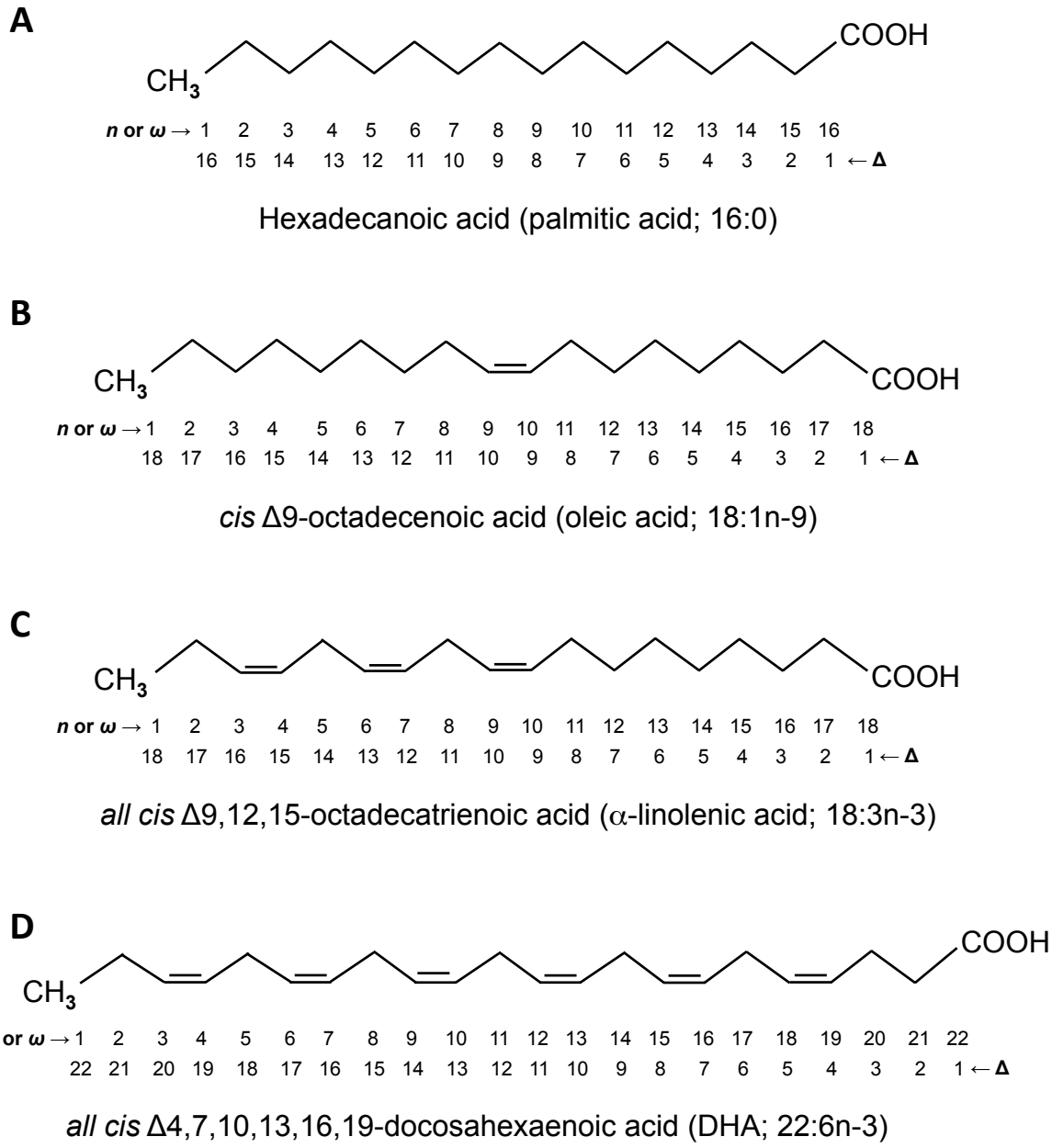
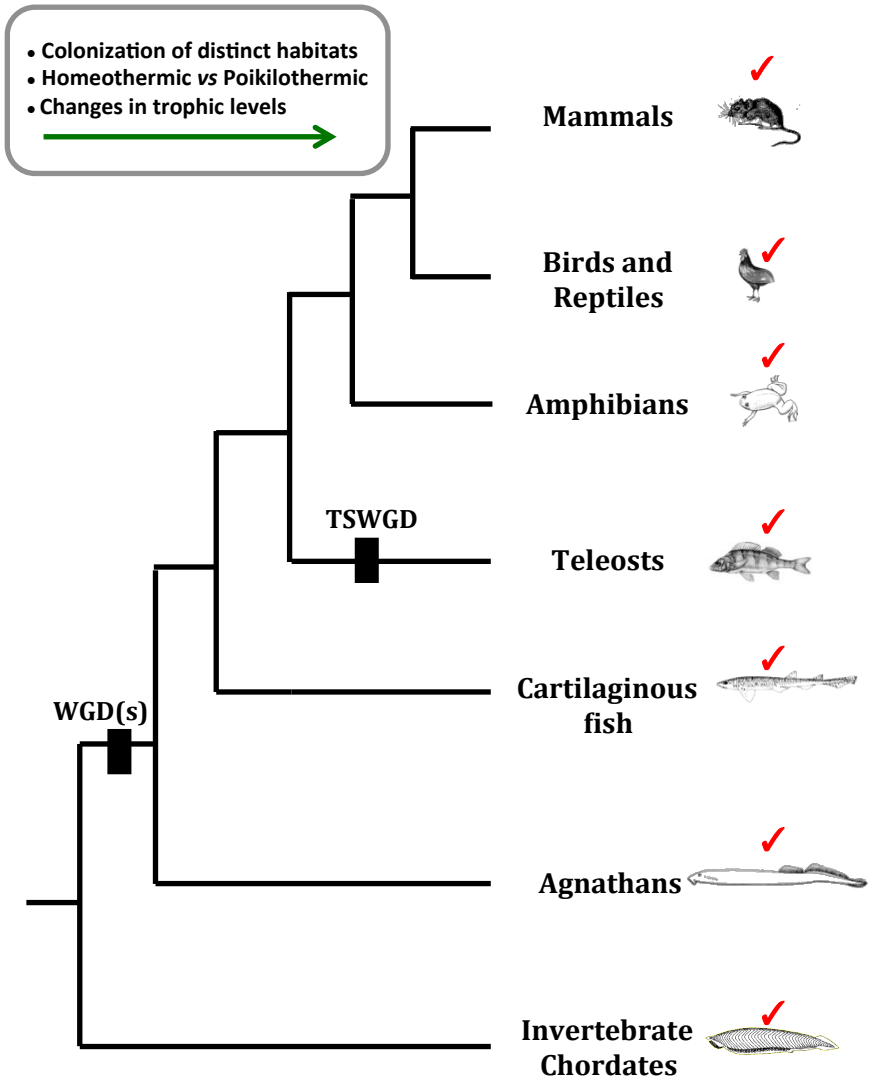


Figure 1. Fatty acid nomenclature. Examples of the A) saturated fatty acid palmitic acid or hexadecanoic acid (16:0), B) monounsaturated fatty acid oleic acid or *cis* Δ9-octadecenoic acid (18:1n-9), C) polyunsaturated fatty acid α-linolenic acid or *all cis* Δ9,12,15-octadecatrienoic acid (18:3n-3), and long-chain polyunsaturated fatty acid docosahexaenoic acid or *all cis* Δ4,7,10,13,16,19-docosahexaenoic acid (22:6n-3).



1494

1495 Figure 2. Phylogenetic relationships of extant chordate lineages. WGD – whole genome
1496 duplications; TSWGD – teleost-specific whole genome duplications. ✓ indicates the
1497 presence of available genome sequence.

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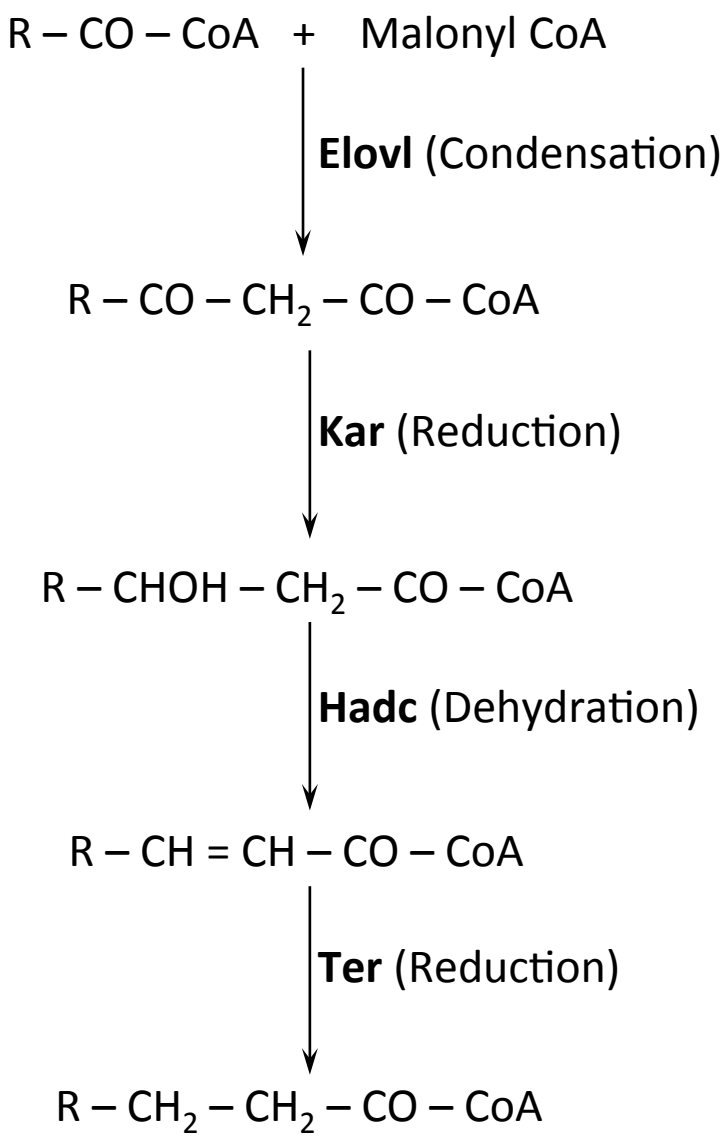


Figure 3. Fatty acid elongation pathway occurring in endoplasmic reticulum. The elongase (Elovl) is responsible for the condensation of malonyl-CoA into an activated fatty acid, regarded as the rate-limiting step in the pathway. Other enzymes involved in the pathway are 3-ketoacyl-CoA reductase (Kar), 3-hydroxyacyl-CoA dehydrase (Hadc) and *trans*-2,3,-enoyl-CoA reductase (Ter).

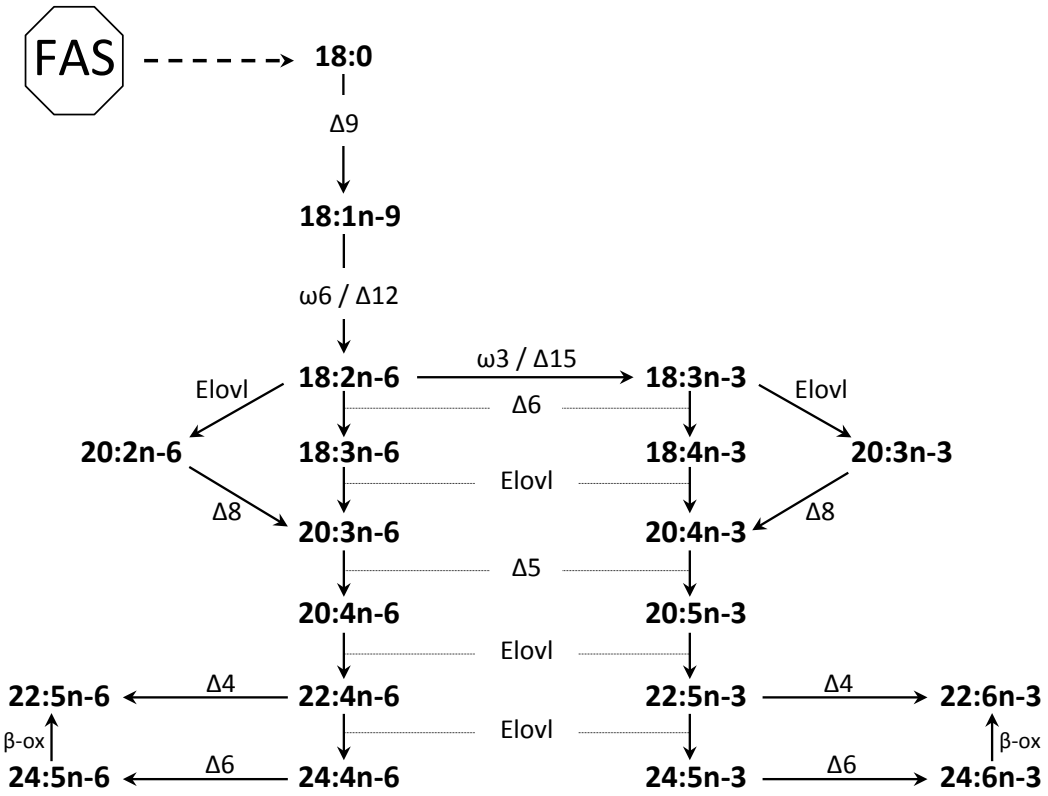
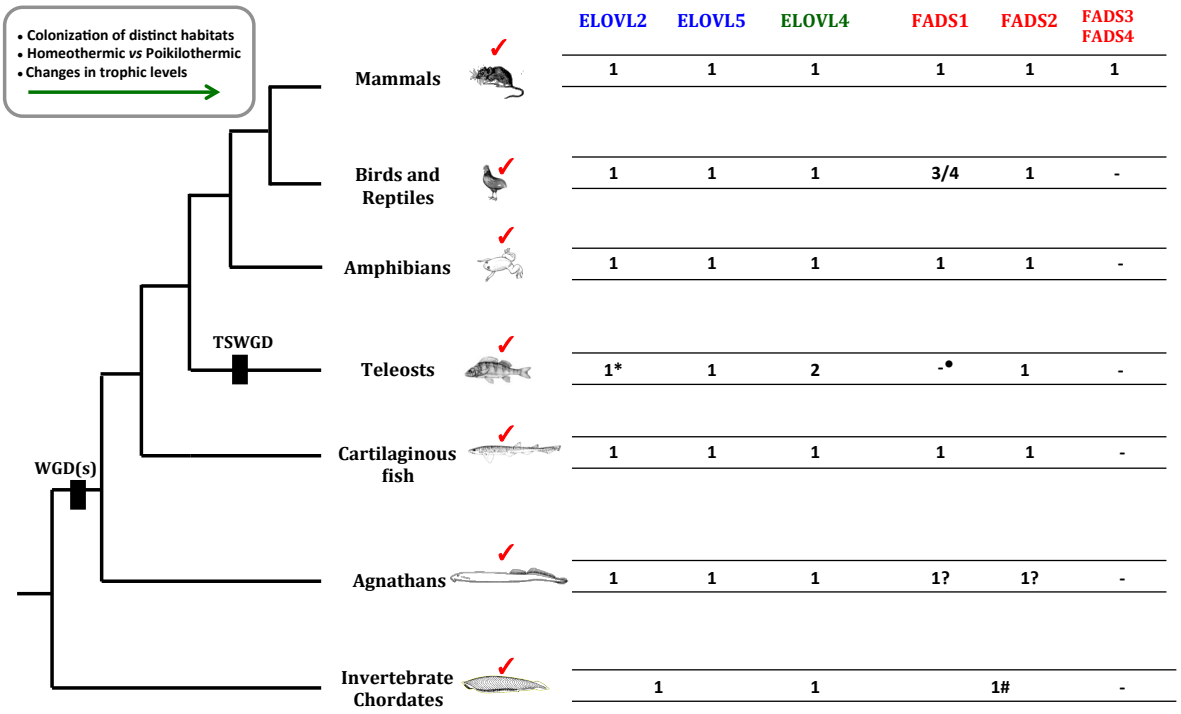


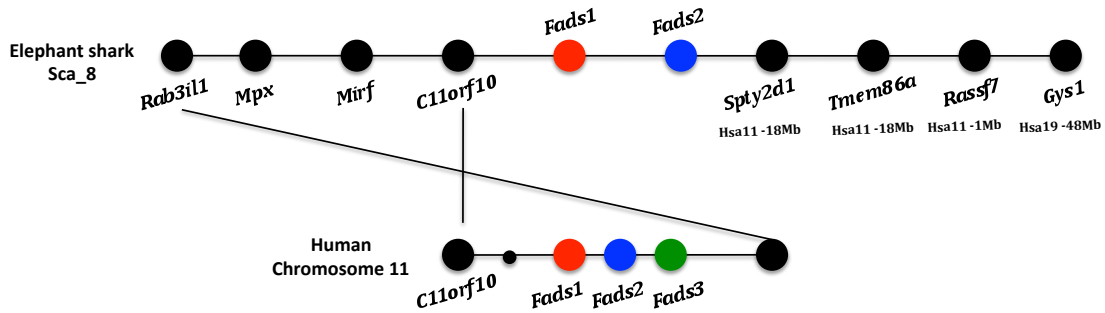
Figure 4. Biosynthetic pathways of polyunsaturated fatty acids. Desaturation reactions are denoted with "ω" or "Δ" to refer, respectively, the carbon position at which the incipient double bond locates within the methyl (ω) and front (Δ) ends of fatty acyl chain. Elongation reactions are catalyzed by Elovl. *De novo* biosynthesis of stearic acid (18:0) by fatty acid synthase (FAS) complex system and subsequent Δ9 unsaturation by stearoyl-CoA desaturase are also shown. Production of 22:6n-3 and 22:5n-6 occurs through partial β-oxidation of 24:6n-3 and 24:5n-6, respectively.



1515

1516 Figure 5. Elovl and Fads gene repertoire in extant chordate lineages. WGD – whole genome
1517 duplications; TSWGD – teleost-specific whole genome duplications. ✓ indicates the
1518 presence of full genome sequences; * indicates gene loss in various teleost species; • indicates
1519 gene loss in species examined to date; # indicates absent gene in tunicates.

1520



1521

1522 Figure 6. The elephant shark Fads *locus*.

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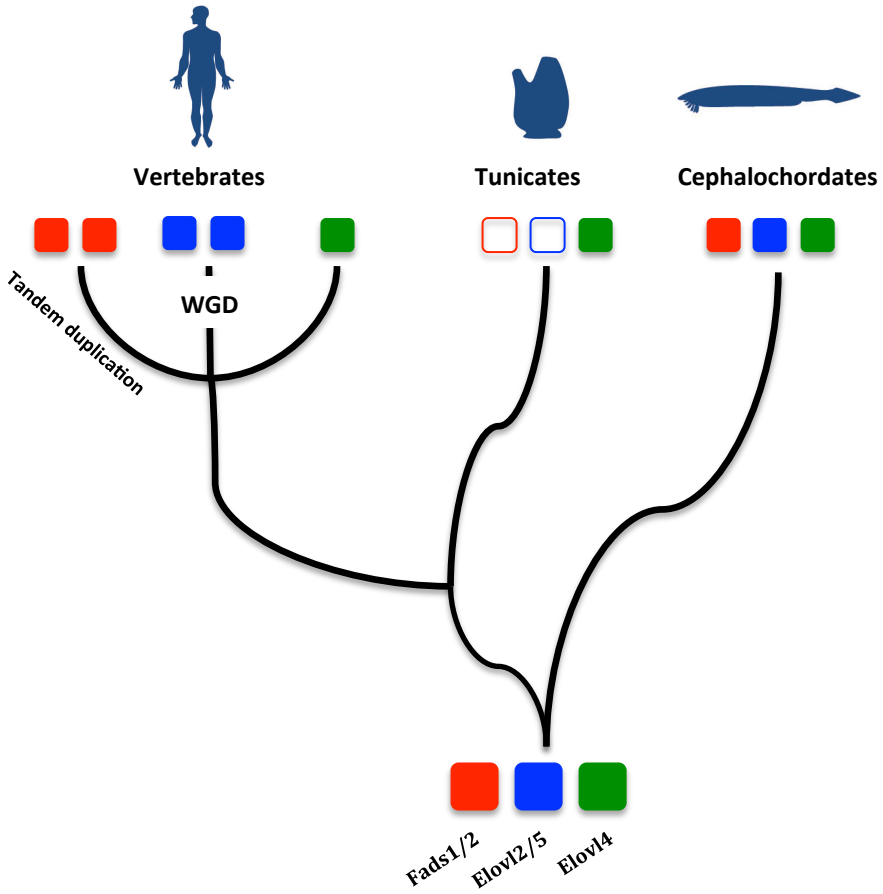

1524

1525 Figure 7. The evolution of Fads and Elovl gene repertoire in chordates. Empty boxes signal

1526 gene loss.

1527

Tables

Table 1. Fatty acyl desaturases investigated in teleosts. The desaturation activities determined for each characterized desaturase enzyme are indicated.

Species*	Common name	Reported activities	References
Danio rerio [1]	Zebrafish	Δ6, Δ5, Δ8	[70, 100]
Oncorhynchus mykiss	Rainbow trout	Δ6, Δ8	[70, 99, 101]
Salmo salar (Δ5Fad)	Atlantic salmon	Δ5	[102]
S. salar (Δ6Fad_a)		Δ6	[103]
S. salar (Δ6Fad_b)		Δ6, Δ8	[70, 104]
S. salar (Δ6Fad_c)		Δ6, Δ8	[70, 104]
Cyprinus carpio	Common carp	Δ6	[101]
Sparus aurata	Gilthead sea bream	Δ6, Δ8	[70, 101, 105]
Psetta maxima	Turbot	Δ6, Δ8	[70, 101]
Gadus morhua	Atlantic cod	Δ6, Δ8	[70, 106]
Rachycentron canadum	Cobia	Δ6, Δ8	[70, 107]
Dicentrarchus labrax	European sea bass	Δ6	[108, 109]
Lates calcarifer	Barramundi	Δ6	[110]
*Siganus canaliculatus (*Fad1*)* [1]	Rabbitfish	Δ6, Δ5, Δ8	[70, 71]
*S. canaliculatus (*Fad2*)* [2]		Δ4, Δ5, Δ8	[70, 71]
Acanthopagrus schlegeli	Black seabream		[111]
Nibea mitsukurii	Nibe croaker	Δ6, Δ8	[112]
Thunnus thynnus	Northern bluefin tuna	Δ6	[113]
Solea senegalensis [2]	Senegalese sole	Δ4, Δ5	[97]
Oreochromis niloticus [1]	Nile tilapia	Δ6, Δ5	[114]
Argyrosomus regius	Meagre	Δ6, Δ8	[98]
*Chirostoma estor (*Fads2a*)* [1]	Pike silverside	Δ4, Δ5	[92]
*C. estor (*Fads2b*)* [2]		Δ6, Δ5, Δ8	[92]
Anguilla japonica	Japanese eel	Δ6, Δ8	[115]
Epinephelus coioides	Orange spotted grouper	Δ6, Δ8	[116]
Channa striata [2]	Striped snakehead	Δ4, Δ5	[117]

*In species with more than one desaturase being studied (*Salmo salar*, *Siganus canaliculatus, Chirostoma estor*), the name of the specific gene is indicated according to published information.

[1] Bifunctional Δ6Δ5 desaturases

[2] Bifunctional Δ4Δ5 desaturases

Table 2. Elongases (Elovl) investigated in teleosts. The type of Elovl is determined by amino acid similarities with mammalian orthologues and substrate specificities as reported in the indicated publications.

Species	Common name	Elongase type	Substrate specificities	References
Danio rerio	Zebrafish	Elovl5	C_{18-22}	[130]
D. rerio		Elovl2	C_{18-24}	[131]
D. rerio		Elovl4a	C_{20-34}	[132]
D. rerio		Elovl4b	C_{20-34}	[132]
Salmo salar	Atlantic salmon	Elovl5a	C_{18-22}	[96, 133, 134]
S. salar		Elovl5b	C_{18-22}	[133, 134]
S. salar		Elovl2	C_{18-22}	[134]
S. salar		Elovl4b	C_{20-34}	[135]
Oreochromis niloticus	Nile tilapia	Elovl5	C_{18-22}	[136]
Clarius gariepinus	African catfish	Elovl5	C_{18-22}	[133]
Sparus aurata	Gilthead sea bream	Elovl5	C_{18-22}	[133]
Psetta maxima	Turbot	Elovl5	C_{18-22}	[133]
Gadus morhua	Atlantic cod	Elovl5	C_{18-22}	[133]
Rachycentron canadum	Cobia	Elovl5	C_{18-22}	[107]
R. canadum		Elovl4b	C_{20-34}	[136]
Lates calcarifer	Barramundi	Elovl5	C_{18-22}	[110]
Nibea mitsukurii	Nibe croaker	Elovl5	C_{18-22}	[112]
N. mitsukurii		Elovl4b	C_{18-22}	[112]
Thunnus maccoyii	Southern bluefin tuna	Elovl5	C_{18-22}	[137]
Thunnus thynnus	Northern bluefin tuna	Elovl5	C_{18-22}	[113]
Siganus canaliculatus	Rabbitfish	Elovl5	C_{18-22}	[138]
S. canaliculatus		Elovl4b	C_{20-34}	[138]
Solea senegalensis	Senegalese sole	Elovl5	C_{18-22}	[97]
Acanthopagrus schlegelii	Black seabream	Elovl5	C_{18-20}	[139]
Argyrosomus regius	Meagre	Elovl5	C_{18-22}	[98]
Esox lucius	Northern pike	Elovl5	C_{18-22}	[96]
Oncorhynchus mykiss	Rainbow trout	Elovl5	C_{18-20}	[140]
O. mykiss		Elovl2	C_{20-22}	[140]
Chirostoma estor	Pike silverside	Elovl5	C_{18-22}	[92]
Anguilla japonica	Japanese eel	Elovl5	C_{18-20}	[115]

Table 3. Fatty acyl desaturases (Fads) and elongases (Elovl) investigated in non-teleost chordates.

Species	Gene	Common name	References
MAMMALS			
Homo sapiens	*FADS1*	Human	[154]
H. sapiens	*FADS2*		[73, 127, 154]
H. sapiens	*ELOVL2*		[142]
H. sapiens	*ELOVL4*		[181]
H. sapiens	*ELOVL5*		[141]
Mus musculus	*Fads1*	Mouse	[156]
M. musculus	*Fads2*		[156]
M. musculus	*Elovl2*		[142]
M. musculus	*Elovl5*		[142]
Rattus norvegicus	*Fads1*	Rat	[155]
R. norvegicus	*Fads2*		[128, 152]
R. norvegicus	*Elovl2*		[146]
R. norvegicus	*Elovl5*		[146, 167]
Papio anubis	*FADS2*	Baboon	[69]
BIRDS			
Gallus gallus	*ELOVL2*	Chicken	[90]
G. gallus	*ELOVL5*		[90]
Anas platyrhynchos	*ELOVL2*	Domestic Mallard duck	[91]
A. platyrhynchos	*ELOVL5*		[91]
Meleagris gallopavo	*ELOVL2*	Australian white hybrid turkey	[91]
M. gallopavo	*ELOVL5*		[91]
TUNICATES			
Ciona intestinalis	*elovl4*	Sea squirt	[88]
CHONDRICHTHYANS			
Scyliorhinus canicula	*fads1*	Lesser-spotted dogfish	[89]
S. canicula	*fads2*	Lesser-spotted dogfish	[89]