Accepted refereed manuscript of:


DOI: [10.1016/j.plipres.2016.01.001](http://dx.doi.org/10.1016/j.plipres.2016.01.001)

© 2016, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International [http://creativecommons.org/licenses/by-nc-nd/4.0/](http://creativecommons.org/licenses/by-nc-nd/4.0/)
Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of Fads and Elovl gene repertoire

L. Filipe C. Castro\textsuperscript{1,2}, Douglas R. Tocher\textsuperscript{3} and Oscar Monroig\textsuperscript{3} *

\textsuperscript{1} CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

\textsuperscript{2} Biology Department, FCUP, University of Porto, Portugal

\textsuperscript{3} Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

*Corresponding author

Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

Tel: +44 1786 467892; Fax: +44 1786 472133; E-mail: oscar.monroig@stir.ac.uk
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 (C20-24) 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 (>C24) 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 (Elov) 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 Elov 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 (C20-24) 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 gene1 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”, the predicted protein as “ELOVL5”; for mouse and rat, gene will be named as “Elov15”, whereas protein will be “ELOV15”; for chicken and other birds, gene will be termed as “ELOVL5”, whereas protein will be “ELOV15”; 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 “Elov15”1; similarly, for zebrafish and other fish, gene will be named as “elovl5”, whereas protein will be “Elov15”. For non-vertebrate organisms and agnathans, we have used the same symbols as described for fish (“elovl5” for genes and “Elov15” for proteins).
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 \( \omega \)x) 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 (18:1\( \omega \)9) 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 (20:5\( \omega \)3), 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 \( \Delta \)x (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\( \Delta \)5,8,11,14,17. Thus, a Fads that introduces an ethylenic (double) bond five carbons from the carboxyl end of the aliphatic chain is described as having \( \Delta \)5 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 \( \beta \)-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, C20 - C24 fatty acids are common and chain lengths > C24 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 b5 domain containing the haem-binding motif, HPGG [34, 39, 40]. Thus, the animal front-end desaturases are fusion proteins containing both desaturase and cytochrome b5 functions [41].

### 2.3.2 Biosynthesis of monounsaturated fatty acids and C18 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 \(\omega6\) 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 \(\omega3\) 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 \(\omega3\) desaturase capable of introducing an \(\omega3\) 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/\(\omega6\) and Δ15/\(\omega3\) 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/\(\omega6\) and Δ15/\(\omega3\) 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\textsubscript{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\textsubscript{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 \textit{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 \textit{Shewanella pneumatophori} SCRC-2738, containing all five pfa genes in close vicinity. Type II, as in \textit{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 \textit{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 \textit{Rhizopus nigricans}, \textit{R. arrhizus}, \textit{Mucor rouxii} and \textit{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\textsubscript{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\textsubscript{18} PUFA. The pathway for the production of LC-PUFA from C\textsubscript{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 \(\Delta 5\) 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 chondrichthyans 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 chondrichthyans 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 chondrichtysans. 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\textsubscript{22} and C\textsubscript{18}, respectively, while a clear overlap was observed for C\textsubscript{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\textsubscript{18} and C\textsubscript{20}, respectively, with no capacity to elongate C\textsubscript{22} PUFA [87]. These results suggested that the agnathans represent an intermediate state after duplication since they completely lack the ability to elongate C\textsubscript{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).
3.2.2 Teleosts

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

3.2.2.1 Fads in teleosts

Teleosts have been by far the group of tetrapods where the greatest number of Fads and Elovl have been studied (Fig. 5). The first Fads-encoding cDNAs were isolated from rainbow trout [99] and zebrafish [100]. The amino acid sequence homology of the two Fads indicated they both were Δ6 desaturases. However, the functional characterization of the zebrafish Fads by heterologous expression in yeast demonstrated that the encoded enzyme was a bifunctional Δ6/Δ5 desaturase [100]. Subsequently, a relatively large number of Fads-like cDNAs have 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]. Interestingly, 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 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 Nbea 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 C24 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
the salmon and trout Δ5 Fads2, other subfunctionalized Fads2 have been subsequently characterized in several fish species.

Arguably the most important and interesting finding in our investigations of teleost LC-PUFA biosynthesis in recent years has been the discovery of a Fads2 in rabbitfish (*Siganus canaliculatus*) with capability for Δ4 desaturation [71], an activity originally believed to account for the production of DHA directly from 22:5n-3 resulting from a single elongation of EPA [68]. More recently Fads2 with Δ4 activities have also been characterized in Senegalese sole *Solea senegalensis* [97], pike silverside *Chirostoma estor* [92] and the striped snakehead *Channa striata* [117]. Further to the isolation and characterization of Δ4 desaturase cDNAs in teleosts, *in vivo* biochemical evidence of the existence of an enzymatic activity enabling Δ4-desaturase-dependent DHA synthesis has been shown recently in *S. senegalensis* [122]. In comparison with the Sprecher pathway, the so-called “Δ4 pathway” is a more direct metabolic route as it avoids translocation of LC-PUFA intermediates (namely 24:6n-3) between endoplasmic reticulum and peroxisomes, and also the further catabolic step (partial oxidation to DHA) occurring in the latter organelle [72, 123]. In addition to the Δ4 Fads2, rabbitfish also expressed a bifunctional Δ6Δ5 Fads2 [71], similar to the zebrafish desaturase, and the high degree of amino acid (aa) sequence similarity between the two rabbitfish Fads2 has prompted investigation of the specific residues dictating the different substrate specificities of Fads2 desaturases [124]. It was concluded that four aa residues (YNYN for Δ4 Fads2 and FHYQ for Δ6Δ5 Fads2) located between the second and third histidine boxes were responsible for the PUFA substrate specificities of each enzyme. *In silico* searches among teleost fish indicated that Fads2 with the key aa residues and thus being putative Δ4 desaturases were more widely spread than initially believed. For instance, cichlids including *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni* and *Pundamilia nyererei*, all possess putative Δ4 Fads2 (Monroig’s personal communication).

As mentioned above, plasticity of teleost Fads2 is not restricted to the existence of monofunctional Δ6, Δ5 or Δ4 desaturases, but some of the enzymes investigated have been shown to be bifunctional proteins. In addition to the abovementioned zebrafish Fads2 that was the first bifunctional Δ6Δ5 desaturase described [100], other bifunctional desaturases were described in fungi [54, 125], protozoans [53] and moths [126]. In fish, the rabbitfish *S. canaliculatus* [71] as described above, Nile tilapia *O. niloticus* and pike silverside *C. estor* [92] also possess bifunctional Δ6Δ5 Fads2 enzymes. These bifunctional desaturases are enzymes that can introduce two separate, distinct double bonds into the same fatty acid chain 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\textsubscript{18} and C\textsubscript{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\textsubscript{18} and C\textsubscript{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\textsubscript{18} and C\textsubscript{20} PUFA, with markedly lower elongation efficiency on C\textsubscript{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\textsubscript{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 C16 PUFA 16:3n-3 [98, 137], 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 C18 substrates (18:4n-3 and 18:3n-6), the Elovl2 were particularly efficient in the elongation of C20 (20:5n-3 and 20:4n-6) and C22
(20:5n-3 and 20:4n-6) LC-PUFA substrates, the latter not regarded as substrates for Elovl5 according to the low (if any) conversions observed in the yeast expression system. Recently, an Elovl2 has been isolated and investigated in rainbow trout O. mykiss [140]. Similar to the observations in S. salar and D. rerio, the O. mykiss Elovl2 showed the ability to elongate C20 and C22 but, in contrast, did not show any activity on C18 PUFA substrates. Interestingly it is unclear whether the ability to elongate C18 PUFA is a common feature among Elovl2 proteins as, like Atlantic salmon and zebrafish orthologues, the mouse ELOVL2 showed some elongation capability on C18 PUFA [141], although the human and chicken did not (see below), similar to rainbow trout Elovl2 [90, 142]. Regardless of its possible role in C18 PUFA elongation, the ability of Elovl2 to elongate 22:5n-3 to 24:5n-3 was regarded as key for the production of DHA via the “Sprecher pathway”, and because of the loss of Elovl2 in the vast majority of marine fish lineages and virtually all commercially important farmed species, this has been hypothesized as a possible factor accounting for the limited ability for DHA biosynthesis in those species [134].

Elovl4 are the most recent member of the Elovl family that has been studied in teleosts. Zebrafish was the first teleost fish species from which Elovl4 were isolated and functionally characterized, representing at that time the only report of a non-human Elovl4 elongase [132]. Two isoforms, termed as Elovl4a and Elovl4b, were described in zebrafish and both showed the ability to efficiently elongate saturated fatty acids up to C36. Interestingly though, activity for the elongation of PUFA substrates was only shown by Elovl4b, which effectively converted the C20 LC-PUFA, EPA and ARA, to elongated products with acyl chain-lengths up to C36. Subsequent studies describing the cloning and functional characterisation of Elovl4 were conducted in cobia (R. canadum), Atlantic salmon (S. salar) and rabbitfish (S. canaliculatus) [135, 136, 138]. In agreement with these enzymes being homologues of the zebrafish Elovl4b isoform, the cobia, Atlantic salmon and rabbitfish Elovl4 enzymes showed the ability to biosynthesize both saturated and polyunsaturated VLC-FA. While Atlantic salmon also possess an Elovl2 with elongation capability towards C22 LC-PUFA [134], the ability of Elovl4 from cobia and rabbitfish, two marine Actinopterygii species, to elongate 22:5n-3 to 24:5n-3 provided evidence to support a role for Elovl4 of some teleosts at least in DHA biosynthesis similar to Elovl2 [136]. More recently, the Nibe croaker Elovl4 [112], another Elovl4b-like orthologue, was shown to be capable of elongating 22:5n-3 to 24:5n-3 although the reported conversions were notably lower (3.6 %) compared to previously reported conversions for zebrafish isoform b (29.8 %), cobia (34.1 %), Atlantic salmon (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
limited ability to elongate C\textsubscript{22} PUFA including 22:5n-3 and, as suggested for the chicken ELOVL5, they can potentially contribute to the biosynthesis of DHA through the Sprecher pathway [72].

The ability of the chicken ELOVL5 to elongate C\textsubscript{22} PUFA does not appear to be a common feature among bird ELOVL5. Thus, a subsequent study on fatty acid elongases of the domestic Mallard duck (\textit{Anas platyrhynchos}) and Australian white hybrid turkey (\textit{Meleagris gallopavo}) showed that the ElovL5 from these species had no activity towards C\textsubscript{22} PUFA including 22:5n-3 and 22:4n-6 [91]. Unlike the chicken ELOVL5, both the duck and turkey ELOVL5 had C\textsubscript{18} PUFA such as 18:4n-3 and 18:3n-6, as well as C\textsubscript{20} PUFA like EPA and ARA as preferred substrates for elongation. Similarly, functional analyses of the duck and turkey ELOVL2 also showed some differences compared to the chicken orthologue [90]. Although the turkey ELOVL2 did not show any activity towards C\textsubscript{18} PUFA substrates like the chicken ELOVL2, the turkey orthologue also showed no activity on ARA that remained unmodified. The duck ELOVL2 was capable of elongating C\textsubscript{20} (including EPA and ARA) and C\textsubscript{22} substrates similar to the chicken ELOVL2 but, in addition, it showed the ability to elongate C\textsubscript{18} PUFA like 18:4n-3 and 18:3n-6, an capability not shown by the chicken orthologue. The ability of ElovL2 proteins to elongate C\textsubscript{18} PUFA substrates to a small extent has also been described in fish as detailed above [131, 134] and mouse [142].

3.4. FADS and ELOVL in mammals

The LC-PUFA biosynthetic enzymes have been extensively studied in human and other mammals, primarily rodent model species such as mouse \textit{Mus musculus} and rat \textit{Rattus norvegicus}. As a result, several reviews have been published in recent years [20, 22, 32]. Therefore, this section will provide a brief overview of the \textit{FADS} and \textit{ELOVL} gene complement in mammals and the functions of their protein products with a role in LC-PUFA biosynthesis. Additionally, we will also discuss the most recent advances on evolutionary aspects of these gene families.

3.4.1 Mammalian FADS

Mammals express \(\Delta_6\) and \(\Delta_5\) desaturases with the ability to desaturate at the \(\Delta_6\) and \(\Delta_5\) positions of PUFA substrates. In humans the \(\Delta_5\) and \(\Delta_6\) desaturase enzymes are encoded by the \textit{FADS1} and \textit{FADS2} genes, respectively [147] (Fig. 5). These genes are organized into a
tight physical cluster on chromosome 11, and a further desaturase, FADS3 with unknown
tight physical cluster on chromosome 11, and a further desaturase, FADS3 with unknown
function [148], is also part of the cluster [147, 148]. Interestingly, Castro and co-workers [89]
also identified a fourth Fads gene (Fads4) sequence occurring at a distant genomic location of
the FADS1/FADS2/FADS3 gene cluster in mouse. The function and role of murine Fads4
remains to be characterized, but its unique phylogenetic distribution and rather restricted
occurrence in some mammalian species, perhaps suggests a very specific role.

By comparison of the sequences of known Δ6 desaturases from a cyanobacterium [150],
nematode [41] and plant [151], full lengths cDNAs for Δ6 FADS2 desaturases were
characterized in mammals including rat [152], mouse and human [153] (Table 3). Functional
characterization demonstrated that the C18 PUFA, 18:3n-3 and 18:2n-6, were adequate
substrates for the mammalian Δ6 desaturases and, subsequently, it was demonstrated that the
Δ6 desaturase from human [127] and rat [128] also had the ability to desaturate 24:5n-3 at the
Δ6 position [127]. While the recent discovery of Δ4 activity of the human FADS2 provides an
alternative and direct pathway for DHA biosynthesis [73], historically it was accepted that
DHA production was achieved through the Sprecher pathway and, thus, FADS2 mediates the
Δ6 desaturation of 24:5n-3 to 24:6n-3, which is thereafter translocated to peroxisomes for
chain shortening via partial β-oxidation [123]. This makes DHA biosynthesis by the Sprecher
pathway a somewhat inefficient process and thus Δ6 desaturase has been regarded as the
overall rate-limiting factor in the LC-PUFA biosynthetic pathway in mammals [22]. Unlike
Δ6 desaturases, the Δ5 desaturases operate at one single step of the pathway, converting
20:4n-3 to EPA in the n-3 series, and 20:3n-6 to ARA in the n-6 series (Fig. 4). Several
mammalian Δ5 FADS1 desaturase genes have been characterized from human [154], rat
[155] and mouse [156]. Interestingly, non-coding RNA genes, which are transcribed from the
opposite strand of the mammalian FADS1 gene, were later discovered in human, mouse and
rat [157]. Such reverse Δ5-desaturase was found to regulate the expression and enzymatic
activity of the rat FADS1, supporting a significant role as a natural antisense regulator of Δ5-
desaturase.

In addition to the above described Δ6 desaturation capability of mammalian FADS2, this
enzyme can also operate as a Δ8 desaturase and thus PUFA substrates including 20:3n-3 and
20:2n-6 can be desaturated to 20:4n-3 and 20:3n-6, respectively, to thus reincorporate them
into the pathway for further Δ5 desaturation [69] (Fig. 4). It is worth mentioning that the Δ8
desaturase capacity of the baboon FADS2 [69] seemed to be notably lower compared to those
of marine teleost Fads2 assayed under similar conditions [70]. These findings represent
further evidence of subfunctionalization occurring in teleost Fads2. Importantly, while the
teleostei Elovl5 has been identified to elongate C_{18} PUFA (18:3n-3 and 18:2n-6) required for
the Δ8 pathway, it still remains to be elucidated if the mammalian ELOVL5 is responsible for
such conversions [22]

Adaptive evolution studies have recently revealed that ability to biosynthesize LC-PUFA
varies notably among human populations and, for instance, levels of LC-PUFA in African-
American individuals were found to be higher compared to European-Americans [158, 159].
Such differences were linked to polymorphisms in the FADS gene cluster that then lead to
higher capacity to biosynthesis LC-PUFA from C_{18} precursors. It was postulated that highly
efficient desaturation alleles of FADS genes are particularly abundant in African populations
where these were retained by positive selection driven by dietary limitation of preformed LC-
PUFA in isolated regions [160].

3.4.1 Mammalian ELOVL

Seven members of the ELOVL protein family (ELOVL 1-7) have been described in
mammals [20, 22]. The elongation of saturated and monounsaturated fatty acids in mammals
can be catalyzed by four elongases, namely ELOVL1, ELOVL3, ELOVL6 and ELOVL7, that
show different tissue distributions probably reflecting different functional roles. ELOVL1 is
thought to be involved in the production of saturated fatty acids up to C_{26} in length, as present
in sphingolipids [161, 162]. It is expressed in most tissues, which may reflect a housekeeping
role and a requirement for these fatty acids and lipids containing them in membranes, but it is
also highly expressed in some parts of the central nervous system and may have an important
role in the synthesis of sphingomyelin and hence myelin [162, 163]. ELOVL3 is suggested to
control the synthesis of saturated and monounsaturated fatty acids of up to C_{24} and is
expressed mainly in brown and white adipose tissue, skin and liver [20, 164]. ELOVL3 is
cold inducible in brown adipose tissue [164], displays a diurnal expression in the liver, and is
regulated by gender-specific steroid hormones such as glucocorticoids, androgens and
oestrogens [165]. ELOVL6 is involved in the elongation of C_{12-16} Saturated fatty acids up to
C_{18}, but it has no capability to elongate beyond C_{18}. ELOVL6 is ubiquitously expressed but
especially in liver, brain, and other tissues with high lipid content such as white and brown
adipose tissue [166, 167]. ELOVL7, the latest member of the ELOVL family to be identified,
is highly expressed in the kidney, pancreas, adrenal glands and prostate [168]. Knockdown
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 Elolv5 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
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.
Acknowledgements
LFCC research is funded by the Foundation for Science and Technology (Strategic Funding UID/Multi/04423/2013).

References


Identification and characterization of an animal Δ(12) fatty acid desaturase gene by heterologous expression in *Saccharomyces cerevisiae*. Arch Biochem Biophys 2000; 376:399–408.

*Caenorhabditis elegans* Δ12-desaturase FAT-2 is a bifunctional desaturase able to desaturate a diverse range of fatty acid substrates at the Δ12 and Δ15 positions. J Biol Chem 2011; 286:43644–50.


Characterization, mRNA expression and regulation of Δ6 fatty acyl desaturase (FADS2) by dietary n – 3 long chain polyunsaturated fatty acid (LC-PUFA) levels in grouper larvae (*Epinephelus coioides*). *Aquaculture* 2014; 434:212–9.


Buzzi M, Henderson RJ, Sargent JR. The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus mykiss*) fed diets containing fish oil or olive oil. Biochim Biophys Acta 1996; 1299:235-44.


Aveldano MI, Robinson BS, Johnson D, Poulos A. Long and very long chain polyunsaturated fatty acids of the n-6 series in rat seminiferous tubules. Active desaturation of 24:4n-6 to 24:5n-6 and concomitant formation of odd and even chain tetraenoic and pentaenoic fatty acids up to C32. J Biol Chem 1993; 268:11663–9.


Furland NE, Oresti GM, Antollini SS, Venturino A, Maldonado EN, Aveldano MI. Very long-chain polyunsaturated fatty acids are the major acyl groups of sphingomyelins and ceramides in the head of mammalian spermatozoa. J. Biol. Chem. 2007; 282:18151-61.


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 \( \text{cis} \Delta 9\)-octadecenoic acid (18:1n-9), C) polyunsaturated fatty acid \( \alpha \)-linolenic acid or \( \text{all cis} \Delta 9,12,15\)-octadecatrienoic acid (18:3n-3), and long-chain polyunsaturated fatty acid docosahexaenoic acid or \( \text{all cis} \Delta 4,7,10,13,16,19\)-docosahexaenoic acid (DHA; 22:6n-3).
Figure 2. Phylogenetic relationships of extant chordate lineages. WGD – whole genome duplications; TSWGD – teleost-specific whole genome duplications. ✓ indicates the presence of available genome sequence.
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.
Figure 5. Elovl and Fads gene repertoire in extant chordate lineages. WGD – whole genome duplications; TSWGD – teleost-specific whole genome duplications. ✓ indicates the presence of full genome sequences; * indicates gene loss in various teleost species; • indicates gene loss in species examined to date; # indicates absent gene in tunicates.

<table>
<thead>
<tr>
<th>ELOVL2</th>
<th>ELOVL5</th>
<th>ELOVL4</th>
<th>FADS1</th>
<th>FADS2</th>
<th>FADS3</th>
<th>FADS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3/4</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>1*</td>
<td>1</td>
<td>2</td>
<td>-*</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1?</td>
<td>1?</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1#</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Colonization of distinct habitats
- Homeothermic vs Poikilothermic
- Changes in trophic levels

Figure 6. The elephant shark Fads locus.
Figure 7. The evolution of Fads and Elovl gene repertoire in chordates. Empty boxes signal gene loss.
Table 1. Fatty acyl desaturases investigated in teleosts. The desaturation activities determined for each characterized desaturase enzyme are indicated.

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

*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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Elongase type</th>
<th>Substrate specificities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Danio rerio</em></td>
<td>Zebrafish</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[130]</td>
</tr>
<tr>
<td><em>D. rerio</em></td>
<td></td>
<td>Elovl2</td>
<td>C\textsubscript{18-24}</td>
<td>[131]</td>
</tr>
<tr>
<td><em>D. rerio</em></td>
<td></td>
<td>Elovl4a</td>
<td>C\textsubscript{20-34}</td>
<td>[132]</td>
</tr>
<tr>
<td><em>D. rerio</em></td>
<td></td>
<td>Elovl4b</td>
<td>C\textsubscript{20-34}</td>
<td>[132]</td>
</tr>
<tr>
<td><em>Salmo salar</em></td>
<td>Atlantic salmon</td>
<td>Elovl5a</td>
<td>C\textsubscript{18-22}</td>
<td>[96, 133, 134]</td>
</tr>
<tr>
<td><em>S. salar</em></td>
<td></td>
<td>Elovl5b</td>
<td>C\textsubscript{18-22}</td>
<td>[133, 134]</td>
</tr>
<tr>
<td><em>S. salar</em></td>
<td></td>
<td>Elovl2</td>
<td>C\textsubscript{18-22}</td>
<td>[134]</td>
</tr>
<tr>
<td><em>S. salar</em></td>
<td></td>
<td>Elovl4b</td>
<td>C\textsubscript{20-34}</td>
<td>[135]</td>
</tr>
<tr>
<td><em>Oreochromis niloticus</em></td>
<td>Nile tilapia</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[136]</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em></td>
<td>African catfish</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[133]</td>
</tr>
<tr>
<td><em>Sparus aurata</em></td>
<td>Gilthead sea bream</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[133]</td>
</tr>
<tr>
<td><em>Psetta maxima</em></td>
<td>Turbot</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[133]</td>
</tr>
<tr>
<td><em>Gadus morhua</em></td>
<td>Atlantic cod</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[133]</td>
</tr>
<tr>
<td><em>Rachycentron canadum</em></td>
<td>Cobia</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[107]</td>
</tr>
<tr>
<td><em>R. canadum</em></td>
<td></td>
<td>Elovl4b</td>
<td>C\textsubscript{20-34}</td>
<td>[136]</td>
</tr>
<tr>
<td><em>Lates calcarifer</em></td>
<td>Barramundi</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[110]</td>
</tr>
<tr>
<td><em>Nibea mitsukurii</em></td>
<td>Nibe croaker</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[112]</td>
</tr>
<tr>
<td><em>N. mitsukurii</em></td>
<td></td>
<td>Elovl4b</td>
<td>C\textsubscript{18-22}</td>
<td>[112]</td>
</tr>
<tr>
<td><em>Thunnus maccoyii</em></td>
<td>Southern bluefin tuna</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[137]</td>
</tr>
<tr>
<td><em>Thunnus thynnus</em></td>
<td>Northern bluefin tuna</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[113]</td>
</tr>
<tr>
<td><em>Siganus canaliculatus</em></td>
<td>Rabbitfish</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[138]</td>
</tr>
<tr>
<td><em>S. canaliculatus</em></td>
<td></td>
<td>Elovl4b</td>
<td>C\textsubscript{20-34}</td>
<td>[138]</td>
</tr>
<tr>
<td><em>Solea senegalensis</em></td>
<td>Senegalese sole</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[97]</td>
</tr>
<tr>
<td><em>Acanthopagrus schlegelii</em></td>
<td>Black seabream</td>
<td>Elovl5</td>
<td>C\textsubscript{18-20}</td>
<td>[139]</td>
</tr>
<tr>
<td><em>Argyrosomus regius</em></td>
<td>Meagre</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[98]</td>
</tr>
<tr>
<td><em>Esox lucius</em></td>
<td>Northern pike</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[96]</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>Rainbow trout</td>
<td>Elovl5</td>
<td>C\textsubscript{18-20}</td>
<td>[140]</td>
</tr>
<tr>
<td><em>O. mykiss</em></td>
<td></td>
<td>Elovl2</td>
<td>C\textsubscript{20-22}</td>
<td>[140]</td>
</tr>
<tr>
<td><em>Chirostoma estor</em></td>
<td>Pike silverside</td>
<td>Elovl5</td>
<td>C\textsubscript{18-22}</td>
<td>[92]</td>
</tr>
<tr>
<td><em>Anguilla japonica</em></td>
<td>Japanese eel</td>
<td>Elovl5</td>
<td>C\textsubscript{18-20}</td>
<td>[115]</td>
</tr>
</tbody>
</table>
Table 3. Fatty acyl desaturases (Fads) and elongases (Elovl) investigated in non-teleost chordates.

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