Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pergamon
Biochemical Systematics and Ecology 31 (2003) 1111–1124
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Evolution of the membrane-bound fatty acid desaturases

D. López Alonso [a,*], F. García-Maroto [b], J. Rodríguez-Ruiz [a], J.A. Garrido [b], M.A. Vilches [a]

[a] *Departamento de Biología Aplicada, Universidad de Almería, La Cañada 04071 Almería, Spain*
[b] *Departamento de Bioquímica, Universidad de Almería, La Cañada 04071 Almería, Spain*

Received 27 May 2002; accepted 3 January 2003

Abstract

The deduced amino acid sequences of the membrane-bound desaturase genes have been compared in order to infer their phylogenetic relationships. All the deduced proteins share three highly conserved histidine rich motifs suggesting a common origin. The phylogenetic analysis revealed three distinct clusters within the membrane desaturases. One cluster consisted of Δ9 desaturase sequences, the second group included the Δ12/ω3 desaturases, and the third cluster comprised the so called 'front-end' desaturases, namely Δ5, Δ6 and Δ8 desaturases. Based on functional data the Δ9 desaturase gene is assumed to be the ancestor of the remaining membrane desaturase genes. The arrangement of the second cluster suggest that ω3 desaturases originated in a prokaryotic lineage from a Δ12 desaturase gene. The first two clusters were essentially consistent with conventional species trees, as the arrangement within the different desaturase classes reflected the evolutionary relationships of the organisms concerned. Conversely, within the 'front-end' desaturase cluster, phylogenetic and functional data indicate that Δ5 desaturase genes originated independently in different evolutionary lineages from an ancestral Δ6 desaturase. In addition, Δ8 desaturases seem to have evolved once in plants from a Δ6 desaturase gene. Available genomic sequences of front-end desaturase genes from higher plants are intronless, while those from lower plants and animals are split. Evolutionary implications of these findings are discussed.

Keywords: Membrane desaturases; Desaturase gene evolution; Intron-less gene; Front-end desaturases

* Corresponding author. Tel.: +34-950-01-5033; fax: +34-950-01-5476.
E-mail address: dlopez@ual.es (D. López Alonso).

0305-1978/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0305-1978(03)00041-3

1. Introduction

Fatty acid desaturases are enzymes responsible for the production of unsaturated (e.g. oleic acid) and polyunsaturated fatty acids (e.g. linoleic acid) (Fig. 1). They are currently the subject of considerable interest as targets for manipulation of transgenic oilseeds (Murphy, 1999; López Alonso and García-Maroto, 2000). Each fatty acid desaturase introduces a double bond at a specific position of the acyl chain (Los and Murata, 1998). Several desaturases position the double bond by 'counting' from the carboxyl end of the molecule. Among them are the heterogeneous class of the Δ9 desaturases, and the so-called 'front-end' desaturases, i.e. Δ6, Δ5 (Napier et al.,

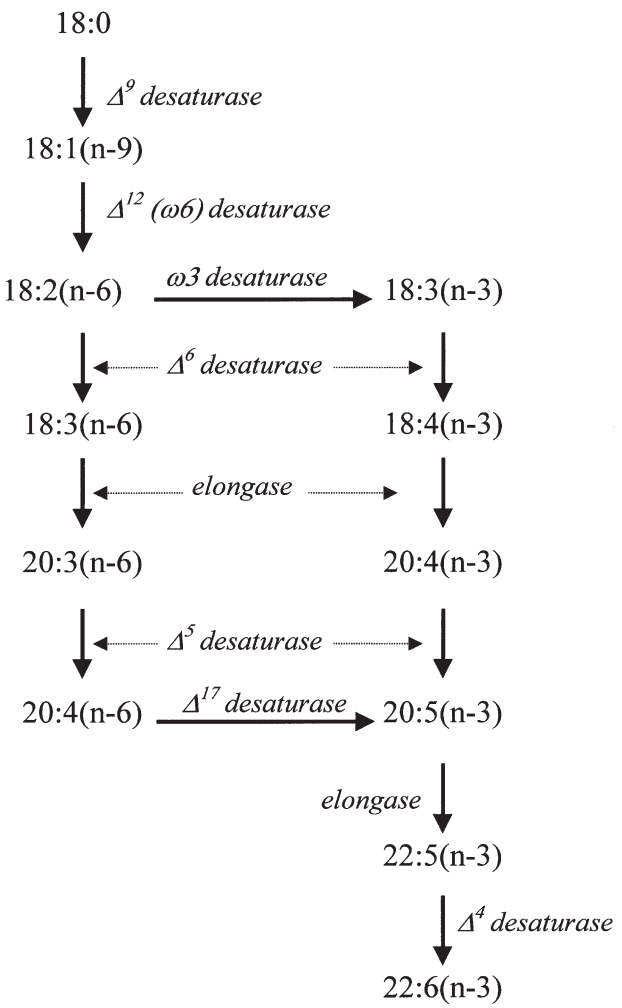

Fig. 1. The biosynthetic pathway of polyunsaturated fatty acids in various organisms.

1999a), and the recently cloned, Δ4 acyl-lipid desaturase (Qiu et al., 2001). In contrast, ω3 desaturases count three carbons from the methyl end of the fatty acid (Higashi and Murata, 1993; Shanklin and Cahoon, 1998) even though they are frequently called Δ15 desaturases. A third desaturase class appears to count three carbons toward the methyl end from an existing double bond. This includes the Δ12 desaturases, also designated as ω6 desaturases (Harwood, 1996; Shanklin and Cahoon, 1998).

Two major classes of desaturases have been described: soluble and membrane-bound desaturases, both of them diiron-oxo enzymes (Murphy, 1999). Soluble desaturases are acyl-acyl carrier protein (ACP) desaturases, represented by the stearoyl-ACP desaturase (EC 1.14.99.6) which desaturates stearoyl-ACP to produce ACP-bound oleic acid (18:1). Membrane desaturases introduce double bonds into fatty acids that are either esterified as acyl-CoA or bound to the glycerol moiety of glycerolipids (Los and Murata, 1998). Soluble desaturases are known to have two conserved histidine motifs (Shanklin and Cahoon, 1998) while membrane desaturases contained three separate histidine-boxes and four transmembrane domains (Los and Murata, 1998; Murphy, 1999). Soluble desaturases are restricted to higher plants and show no evolutionary relationship with the more widely distributed membrane desaturases (Somerville and Browse, 1996; Shanklin and Cahoon, 1998).

Although there are some fragmentary data, a comprehensive analysis of the phylogenetic relationships among different desaturases is missing. In this work we have focused on the genealogy of the membrane fatty acid desaturases since they share a common evolutionary origin. We have also included the Δ8 sphingolipid desaturases since they are highly related to Δ6 fatty acid desaturases and have been proposed as the ancestor of the remaining 'front-end' desaturases (Napier et al., 1999a, 1999b). Recently, it has been conclusively shown the existence of the disputed Δ4 fatty acid desaturase (Qiu et al., 2001) that is not considered in this study because it is by now the only representative of the Δ4 desaturase group.

2. Materials and methods

The *Echium* Δ6 desaturase sequences have been recently reported by us (García-Maroto et al., 2002). All the other sequences were retrieved from the GenBank (National Center for Biotechnology Information, Bethesda, MD) and are listed in Table 1. Sequences were selected trying to cover most of the living world and avoiding 'redundancy' for each desaturase. Thus, when closely related versions of a given desaturase, likely representing gene copies, were present in an organism, only one representative gene was considered. This was the case of the Δ8 and Δ9 desaturases of *Arabidopsis* or the human Δ6 desaturases, for example. Amino acid sequences were aligned using the CLUSTAL X v. 1.81 program (Thompson et al., 1997). The final alignment was further refined after excluding the poorly conserved regions at the protein ends, and consisted of 390 positions spanning the three histidine boxes. This is available from the authors upon request.

The alignment was used to generate a preliminary Neighbor-Joining unrooted tree

Table 1
List of organisms and membrane desaturase protein sequences analysed in this study. The list is ordered by the labels

Organism	Accession no.	Label
Arabidopsis thaliana	AAA32782	d12Arabido
Arachis hypogea	AAB84262	d12Arachis
Aspergillus nidulans	AAG36933	d12Aspergi
Borago officinalis	AAC31698	d12Borago
Brassica juncea	CAA62578	d12Brassic
Arabidopsis thaliana	AAA92800	d12chlArab
Brassica napus	AAA50157	d12chlBras
Glycine max	AAA50158	d12chlGlyc
Glycine max	AAB00860	d12Glycine
Gossypium hirsutum	CAA65744	d12Gossypi
Mortierella alpina	AAF08684	d12Mortier
Mucor circinelloides	AAD55982	d12Mucor
Synechococcus sp.	BAA02922	d12Synechoco
Vernonia galamensis	AAF04094	d12Vernoni
Caenorhabditis elegans	AAC95143	d5Caenorha
Dictyostelium discoideum	BAA37090	d5Dyctiost
Homo sapiens	AAF29378	d5Homo
Limnanthes douglasii	AAG28599	d5Limnanth
Mortierella alpina	AAC39508	d5Mortiere
Rattus norvegicus	AAG35068	d5Rattus
Borago officinalis	AAD01410	d6Borago
Caenorhabditis elegans	AAC15586	d6Caenorha
Danio rerio	NP_571720	d6Danio
Echium gentianoides	AAL23580	d6Echiumge
Echium pitardii	AAL23581	d6Echiumpi
Homo sapiens	AAD20018	d6Homo
Mortierella alpina	AAF08685	d6Mortiere
Mucor circinelloides	BAB69055	d6Mucor
Mus musculus	NP_062673	d6Mus
Oncorhynchus mykiss	AAK26745	d6Oncorhyn
Physcomitrella patens	CAA11033	d6Physcomi
Rattus norvegicus	BAA75496	d6Rattus
Spirulina platensis	CAA60573	d6Spirulin
Synechocystis sp.	AAA27286	d6Synechocy
Arabidopsis thaliana	CAA11858	d8Arabidop
Borago officinalis	AAG43277	d8Borago
Brassica napus	CAA11857	d8Brassica
Helianthus annuus	CAA60621	d8Helianth
Achaeta domesticus	AAK25797	d9Achaeta
Arabidopsis thaliana	BAA25181	d9Arabidop
Caenorahabditis elegans	AAF97550	d9Caenorha
Cryptococcus curvatus	CAA71448	d9Cryptoco

(*continued on next page*)

Table 1 (*continued*)

Organism	Accession no.	Label
Cyprinus carpio	CAB57858	d9Cyprinus
Drosophila simulans	CAB52475	d9Drosphi
Gallus gallus	CAA42997	d9Gallus
Helicoverpa zea	AAF81790	d9Helicove
Homo sapiens	XP_005719	d9Homo
Mortierella alpina	CAB38177	d9Mortiere
Pichia augusta	BAA11837	d9Pichia
Planotortrix orto	AAF73073	d9Planotor
Rattus norvegicus	NP_114029	d9Rattus
Rosa (hybrid cultivar)	AAB50679	d9Rosa
Saccharomyces cerevisiae	AAA34826	d9Saccharo
Spirulina platensis	CAA05166	d9Spirulin
Synechococcus sp.	AAB61353	d9Synechoco
Synechocystis sp.	CAA37584	d9Synechocy
Trichoplusia ni	AAB92583	d9Trichopl
Arabidopsis thaliana	BAA05514	w3Arabidop
Caenorhabditis elegans	AAA67369	w3Caenorha
Arabidopsis thaliana	AAB60302	w3chlArabi
Brassica juncea	CAB85467	w3chlBrass
Picea abies	CAC18722	w3chlPicea
Triticum aestivum	BAA28358	w3chlTriti
Nicotiana tabacum	BAA05515	w3Nicotian
Oryza sativa	BAA11397	w3Oryza
Ricinus communis	AAA73511	w3Ricinus
Solanum tuberosum	CAA07638	w3Solanum
Synechococcus sp	AAB61352	w3Synechoco
Synechocystis sp.	BAA02924	w3Synechocy
Vernicia fordii	CAB45155	w3Vernicia

that justified the use of Δ9 desaturases as the outgroup in the other analysis. The neighbor-joining (Saitou and Nei, 1987) analysis was performed with the MEGA v. 2.1 software package (Kumar et al., 2001) using Poisson corrected distances and ignoring deletions in pairwise comparisons. The tree was rooted using the Δ9 desaturase cluster as the outgroup. Bootstrap (Felsenstein, 1985) with 500 replicates was performed to establish the confidence limit of the tree branches. Parsimony procedures were carried out using the PHYLIP v. 3.6 (Phylogeny Inference Package) software (Felsenstein, 2001). Bootstrap was performed in 100 replicates on the alignment file, and the resulting file was fed to the PROTPARS protein parsimony utility under the multiple data set option. The consensus tree was generated by the CONSENSE program with the majority rule option, and bootstrap values were assigned to each node.

3. Results

The full alignment of the amino acid sequences showed that all membrane desaturases shared the three typical histidine rich motifs (Table 2) that are likely involved in the catalysis of the desaturation reaction. Moreover, there is a constant conservation of several residues within these histidine boxes (Table 2) that is consistent with a common evolutionary origin for all membrane desaturases, including Δ9 desaturases.

The phylogenetic relationships between membrane desaturase genes were analysed using both neighbor-joining (NJ) and parsimony (PA) methods in an effort to determine the patterns of evolutionary diversification. Amino acid sequences encompassing the three histidine boxes and excluding the less conserved protein ends were used in these analysis. In order to place a root in the resulting trees a hypothesis was made about Δ9 desaturases as being the most ancestral group. This assumption was mainly based on functional criteria, as all other desaturases require the existence of a previous double bond at the Δ9 position of the fatty acid that acts as a substrate (Fig. 1). This notion is also supported by the universality of the Δ9 desaturase genes which are represented in all living organisms tested so far, in contrast to the other desaturase classes (see Discussion). In addition, observation of the unrooted NJ tree showed that Δ9 desaturases clustered in a single monophyletic group (not shown), and were therefore taken as the outgroup in further analysis. Fig. 2 shows a parsimony-based phylogenetic tree depicting the relationships among the different membrane desaturases. Supports are estimated at the different nodes from the results of 100 bootstrap replicates. The topology of this tree agrees fairly well with the phylogenetic tree derived using the NJ procedure, based on sequence distances calculated by the Poisson corrected method and calculated bootstrap values over 500 replicates. (Fig. 3). Membrane desaturase genes appear organised into three well resolved monophyletic groups. These groups are, the Δ9 desaturase group, a second group comprising Δ12 plus ω3 desaturases; and a functionally heterogeneous group including the so-called ‘front-end’ desaturases, that is, Δ5, Δ6, and Δ8 desaturases (Figs. 2 and 3).

Table 2
Conserved motifs in the membrane bound desaturases

	H-Box 1	H-Box 2	H-Box 3
delta-8	GHDSGHY	WWKWTHNAHH	QLEHHLFP
delta-6	XHDXGHX	WWXXXHXXHH	QXEHHLFP
delta-5	XHDXGHX	WXXXXXXXHH	QXXHHLFP
delta-12[a]	AHECGHXA	XWKXSHXXHHXXTG	HVXHHXFS
delta-12[b]	GHDCXHXS	XWRXXHXXHHXXTN	HXPHHXXX
omega-3[a]	GHDCGHGSFS	XWRXSHRTHHXNXG	HVXHHXFXQ
omega-3[b]	GHDCGHGSFS	XWRXSHRTHHXNXG	HVXHHXFXQ
delta-9	HRXXXH	XWXXXHRXHH	HNXHHXF

[a] Microsomal
[b] Plastidial

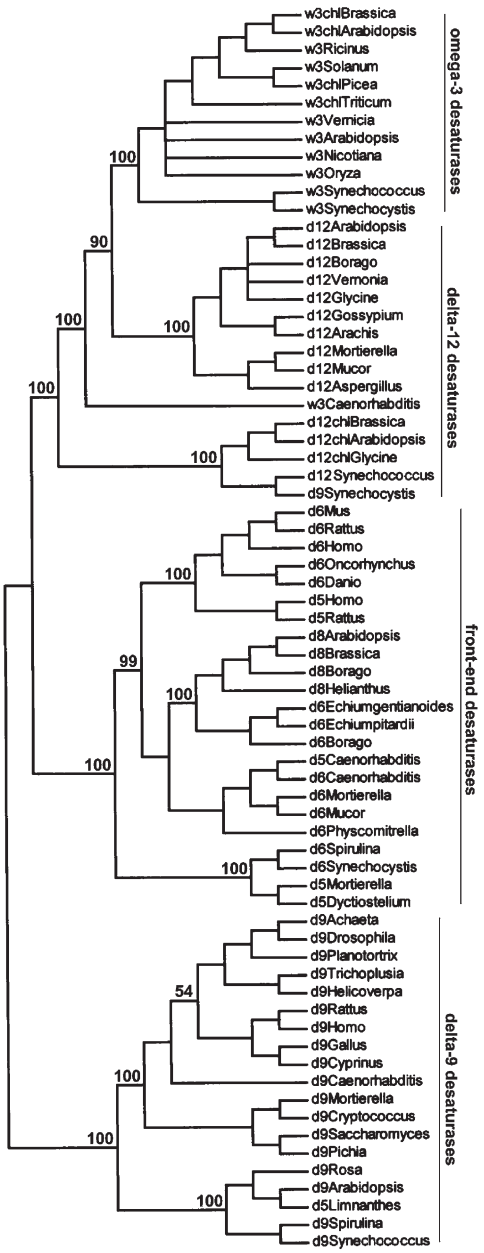

Fig. 2. Neighbor-joining tree showing the relationships among membrane desaturases. Bootstrap values are showed before relevant nodes. Branches with bootstrap support below 50% has been collapsed.

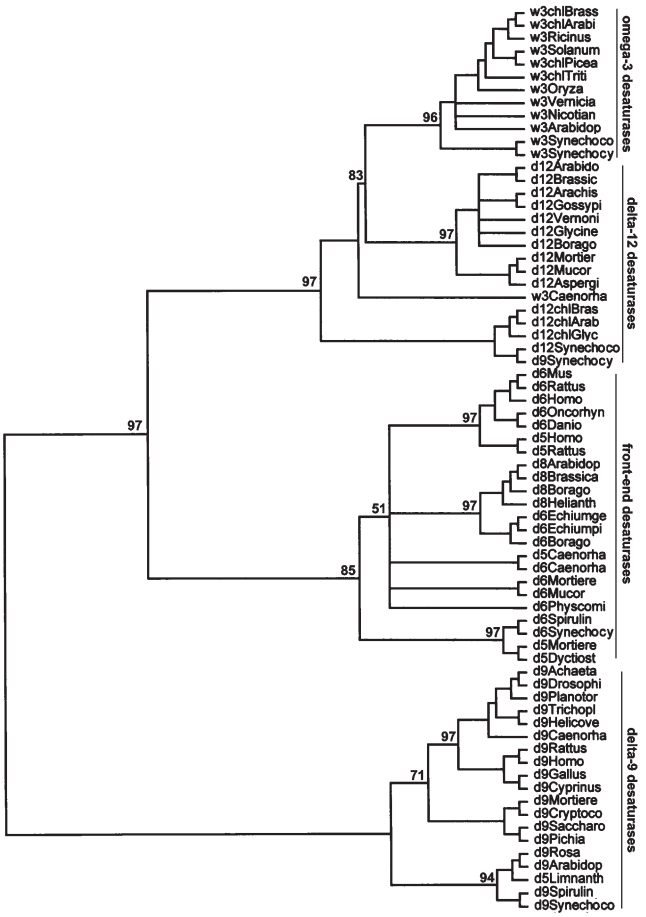

Fig. 3. Parsimony tree showing the relationships among membrane desaturases. Bootstrap values are showed before relevant nodes. Branches with bootstrap support below 50% has been collapsed.

The membrane Δ9 desaturase cluster includes members of cyanobacteria, higher plants, fungi, invertebrates and vertebrates accordingly to its wide phylogenetic distribution. Δ9 desaturases from higher plants are grouped to those from cyanobacteria, in a separate lineage from fungi, and animals. A discrepancy exists in which the Δ9 desaturase of *Synechocystis* appears grouped with the Δ12 desaturases of other cyanobacteria (Fig. 2). It is likely that the initial functional assignation to this protein, which was just based on sequence homology (Kaneko et al., 1996), was wrong, as indicated by the report in the related *Synechococcus* of a closely similar Δ12 desaturase. Moreover, the three histidine-boxes of this desaturase (GHDCGHRS, WRLLHDHHHLHTN, and HIPHH) conform the standards for a prokaryotic Δ12 desaturase (Table 2). Therefore, it is likely that the true function of this *Synechocystis* desaturase is Δ12 instead of Δ9 desaturase. Another apparent ‘anomaly’ that can be

observed in the Δ9 group was the presence of the Δ5 desaturase of *Limnanthes* that, as it will be later discussed, reveals in fact the evolutionary origin of this enzyme.

Monophyly of the Δ12 and the ω3 desaturase group suggests a common origin of both kinds of desaturases. The closest similarities between their respective histidine-boxes is also in agreement to that notion (Table 2). Three different lineages can be distinguished in the Δ12/ω3 desaturase group. The most basal group is integrated by the Δ12-desaturases of cyanobacteria and the chloroplastic Δ12 desaturases of higher plants. This group is supported by very high bootstrap values, 100 in PA analyses, and 97 in the NJ method. A second gene cluster groups the microsomal Δ12 desaturases of fungi and higher plants. A third group includes the ω3 desaturases of cyanobacteria which are placed in a basal position, and both microsomal and chloroplastic ω3 desaturases of higher plants. In this case, the chloroplastic ω3 desaturases of higher plants are not grouped with those from cyanobacteria. As it will be discussed, the position of the ω3 desaturase of *Caenorhabditis* out of this cluster is atypical as it also occurs for other desaturases from this organism.

The 'front-end' desaturase cluster is integrated by four subgroups supported by high bootstrap values in the PA analysis (Fig. 2). The most basal clade is composed by Δ6 desaturases of cyanobacteria and Δ5 desaturases of fungi and the protist *Dictyostelium*. Further analysis reveals that within the same clade are also included the recently cloned Δ4 desaturase of *Thraustochytrium* and the Δ5 desaturases of the diatom *Phaeodactylum* and the oomycete *Phytium*, all of them belonging to the Stramenopile eukaryotic lineage (not shown). A second clade is composed by Δ5 and Δ6 desaturases from vertebrates. Another cluster is integrated by Δ6 and Δ8 desaturases of higher plants which, in turn, constitute clearly separated lineages supported by high bootstrap values. The last clade generated by PA analysis groups Δ6 desaturases of fungi, the moss *Physcomitrella*, and the nematode *Caenorhabditis*, and the Δ5 desaturase of this last organism. This clade is however not supported in neighbor-joining analysis, where the desaturases of fungi, *Caenorhabditis* and *Physcomitrella* appear in separated clusters (Fig. 3). The other clades within the 'front-end' group exhibit high bootstrap values also in the NJ tree.

4. Discussion

The phylogenetic relationships among the different membrane desaturases have been analysed in this work. Knowledge of the enzymatic reactions and the corresponding metabolic pathways, as well as the growing number of desaturase genes cloned from different organisms, allows the formulation of some starting hypotheses and provides a basis for the interpretation of the results. The Δ9 desaturase is the only universally spread desaturase being present in all living beings groups. The remaining desaturases are missing in some of the evolutionary lineages. Active Δ12 and ω3 desaturases are not present in most animals and, consequently, they require an external supply of the so-called 'essential fatty acids' (e.g. linoleic acid, 18:2n6, and α-linolenic acid, 18:3n3). Moreover, a search for putative Δ12 and ω3 desaturases in human, mouse, zebrafish, fruit fly and yeast databases gives a negative result.

The Δ6 desaturases seem to be absent, at least in an active form, in most of higher plants although they are widely distributed in other organisms. A Δ6 desaturase homologous gene is lacking in the *Arabidopsis* genome, suggesting that this could also be the case for other plants which are not able to synthesise γ-linolenic acid (18:3n6, see Fig. 1). Finally, a Δ5 desaturase activity is not present in higher plants and cyanobacteria, although it is found in fungi and animals. The whole picture suggests that, except for the Δ9 desaturase activity which seems to be essential for life, functions performed by the remaining desaturases could have been dispensable in some organisms being replaced along the evolution. On the other hand, the evolution of the Δ12, ω3 and 'front end' desaturases may have required the pre-existence of an enzymatic activity (Δ9 desaturase) able to synthesise the fatty acid (18:1n9) which acts as the initial substrate for those desaturases (Fig. 1). Both arguments support the notion that a Δ9 desaturase gene was the ancestor of the remaining membrane desaturases.

There is an apparent discrepancy with the clustering of the Δ5 desaturase of *Limnanthes* within the Δ9 desaturase group (Fig. 2). However, the Δ5 desaturase of *Limnanthes* is not functionally equivalent to a 'front-end' Δ5 desaturase because its substrate is not 20:3n6, and it does not produce 20:4n6 (Fig. 1); its substrate is acyl-CoA and the product is a Δ5 monounsaturated fatty acid (Cahoon et al., 2000). Therefore, this 'Δ5' desaturase is functionally similar to a Δ9 desaturase except for the position where the double bond is introduced. There are similar enzymes sharing these catalytic features: first, the substrate is a saturated acyl chain, and second, they produce a monounsaturated acyl chain. The differences among these enzymes rely simply on the position of the double bond generated, i.e. Δ4, Δ5, Δ7, etc. (Harwood, 1996). It seems likely that all these enzymes share a common evolutionary origin derived from the Δ9 desaturase lineage.

In relation to the Δ12/ω3 desaturase group, the basal position of the cluster integrated by prokaryotic and chloroplastic Δ12 desaturases (Figs. 2 and 3) indicates that Δ12 desaturases are ancestral to the ω3 desaturases. This makes perfect sense from the functional point of view, since the substrate of the ω3 desaturase is the 18:2n6 (linoleic acid), which is produced from the 18:1n9 by Δ12 desaturation (Fig. 1). The topology of this cluster (with prokaryotic and plant ω3 desaturase genes grouped together in an internal position) is also consistent with the notion that the divergence between Δ12 and ω3 desaturases took place in the prokaryotic lineage, before the appearance of the green plants. This would provide an explanation to the fact that eukaryotic ω3 desaturases seem to be almost restricted to the plants. The close similarity between the plastidial Δ12 desaturases and those from cyanobacteria is consistent with the endosymbiotic origin of this gene, as it should also be the case of the plastidial ω3 desaturase. The origin of the microsomal ω3 desaturase should have taken place by a further recruitment from the procaryotic version of the gene, as indicated by the grouping of both microsomal and chloroplastic genes. The ω3 desaturase gene of *Caenorhabditis* (*FAT-1*) represents the only representative of this class described in animals. However there are evidences suggesting a different evolutionary origin for this desaturase. First, the position of *FAT-1* in the cluster is somewhat odd, as it does not group clearly neither with the ω3 nor with Δ12 desaturases

(Figs. 2 and 3). In addition, its substrate specificity is different to that of plant enzymes, as it is able to desaturate efficiently polyunsaturated long chain fatty acids, such as AA (Spychalla et al., 1997). Finally, and contrary to other ω3 desaturases, *FAT-1* is an intronless gene. Therefore, although functionally homologous to plant ω3 desaturases, FAT-1 might be the result of a convergent evolution. Another remarkable point is the absence of Δ12 desaturases in animals. It can be envisaged that the availability of exogenously supplied fatty acids (i.e. linoleic acid) through the diet could have contributed to dispensability and gene loss in this evolutionary lineage.

The 'front-end' desaturases (Δ5, Δ6 and Δ8 desaturases) cluster separately, thus indicating a common origin (Figs. 2 and 3). This is additionally supported by the presence of a characteristic N-terminal cytochrome b_5 domain, and the sequence of the third histidine box where the first residue is glutamine instead of histidine (Table 2) (Napier et al., 1999a; Sayanova et al., 2001). Similar features are present in the recently reported Δ4 desaturase of *Thraustochytrium* (Qiu et al., 2001). Contrary to other desaturase classes, 'front end' desaturases appear as an heterogeneous group where the precise lineages for Δ5 and Δ6 desaturases cannot be drawn. Based on functional criteria the Δ5 desaturase genes should have originated from a Δ6 desaturase ancestor, since the substrate of the Δ5 desaturase is produced by Δ6 desaturase (Fig. 1). The observation that Δ5 and Δ6 desaturases from vertebrates were more similar than the Δ6 desaturases from plant and vertebrates, or the close similarity between Δ5 and Δ6 desaturases of *Caenorhabditis* (Figs. 2 and 3), suggest that the Δ5 desaturase genes may have arisen independently several times during evolution. Since both desaturase genes reside in the same chromosome and relatively close in humans (Cho et al., 1999) and *Caenorhabditis* (Michaelson et al., 1998), it has been suggested that Δ5 desaturase gene originated from the duplication of the Δ6 desaturase gene followed by further divergence (Michaelson et al., 1998). Recently, it has been reported that the enzyme of zebrafish is able to catalyse both Δ5 and Δ6 desaturase reactions (Hastings et al., 2001). This, together to the close similarity between the *Caenorhabditis* enzymes, supports the notion that the enzyme conversion (i.e. change of specificity) can be achieved through few structural changes. Another interesting point is the grouping of prokaryotic Δ6 desaturases with Δ5 desaturases of lower eukaryotes, such as *Mortierella*, *Dyctiostelium*, and several Stramenopiles. We do not have a clear explanation for that, since some of these organisms lack chloroplasts. One should appeal to more speculative scenarios such as horizontal transfer plus gene conversion.

Δ8 desaturases are only present in higher plants but they appear to be wider distributed than Δ6 desaturases in plants. Based on this fact, it was suggested that the Δ8 desaturases could be ancestral to Δ6 and Δ5 desaturases (Napier et al., 1999a). Nevertheless, if the scope is extended to other kingdoms the conclusion is just opposite: the Δ8 desaturase is restricted to higher plants while the Δ6 and Δ5 desaturases are widely distributed in nature. Therefore, our suggestion is that the Δ8 desaturase originated in plants from a Δ6 desaturase gene. It is possible that Δ6 desaturases were progressively lost in plants, since the ω3 desaturase could supply its function by producing a similar (the alpha isomer) fatty acid (Fig. 1).

Another aspect that is relevant to the evolutionary history of the 'front end' desaturases is the interrupted/continuous nature of the gene. Although we are limited by the low number of genes for which genomic sequences are available, some interesting points can still be addressed. The isolation of the genomic sequences of two Δ6 desaturases of *Echium* (García-Maroto et al., 2002) revealed that the genes were intronless. The available sequences for the two genes encoding Δ8 desaturases in *Arabidopsis*, as well as partial sequences of Δ6 and Δ8 desaturases of *Borago*, and the Δ8 desaturase of *Echium* (unpublished results), indicate that the continuous structure of the gene may be a general feature of Δ6 and Δ8 desaturase genes of higher plants. This is in sharp contrast with the gene structure of the remaining Δ6 desaturases since all reported genes, up to date, were 'fragmented'. This is the case of genes from the moss *Physcomitrella* (Girke et al., 1998), *Homo* (Cho et al., 1999) and *Caenorhabditis* (Napier et al., 1998). The intronless nature of higher plant genes poses two questions, as to when and how they originated. It is possible that the ancestral 'front end' desaturase gene was continuous and that in some evolutionary lineages, like those of animals and lower plants, introns were acquired. Alternatively 'lost' of introns from a discontinuous ancestral gene could have occurred in the evolutionary lineage leading to higher plants. In this case 'retroposition', that is, the insertion of a DNA gene originated from the retrotranscription of a Δ6 desaturase mRNA, might be the underlying mechanism (Brosius, 1999). A third possibility, in which 'horizontal transfer' from intronless genes of other organisms like cyanobacteria might have occurred, seems less likely since Δ6 desaturases of cyanobacteria appear in a separated cluster to that of 'front end' plant desaturases (Figs. 2 and 3).

It is also remarkable that several membrane desaturases contain a cytochrome b_5 domain either on the C-terminus, like in the fungal Δ9 desaturases (Mitchell and Martin, 1995) or on the N-terminus, like in the 'front-end' desaturases (Napier et al., 1999a). The independent acquirement of a cytochrome b_5 domain in different evolutionary branches may have provided a selective advantage by increasing the efficiency of the electron transport required during the unsaturation reaction (Shanklin and Cahoon, 1998).

Acknowledgements

This work was supported by an UAL-CAJAMAR research project and by the Plan Andaluz de Investigación (PAI-III, CVI-279).

References

Brosius, J., 1999. RNAs from all categories generate retrosequences that may be exapted as novel genes or regulatory elements. Gene 238, 115–134.

Cahoon, E.B., Marillia, E.-F., Stecca, K.L., Hall, S.E., Taylor, D.C., Kinney, A.J., 2000. Production of fatty acid components of meadowfoam oil in somatic soybean embryos. Plant Physiol 124, 243–251.

Cho, H.P., Nakamura, M., Clarke, S.D., 1999. Cloning, expression, and fatty acid regulation of the human Δ5 desaturase. J. Biol. Chemistry 274, 37335–37339.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstraps. Evolution 39, 783–791.

Felsenstein, J., 2001. PHYLIP Phylogeny Inference Package 3.6. Department of Genetics, The University of Washington, USA.

García-Maroto, F., Garrido-Cárdenas, J.A., Rodríguez-Ruiz, J., Vilches-Ferrón, M., Adam, A.C., Polaina, J., López Alonso, D., 2002. Cloning and molecular characterization of the Δ6-desaturase from two *Echium* plant species: production of γ-linolenic acid by heterologous expression in yeast and tobacco. Lipids 37, 417–426.

Girke, T., Schmidt, H., Zähringer, U., Reski, R., Heinz, E., 1998. Identification of a novel Δ6 acyl-group desaturase by targeted gene disruption in *Physcomitrella patens*. Plant J 15, 39–48.

Harwood, J.L., 1996. Recent advances in the biosynthesis of plant fatty acids. Biochim. Biophys. Acta 1301, 7–56.

Hastings, N., Agaba, M., Tocher, D.R., Leaver, M.J., Dick, J.R., Sargent, J.R., Teale, A.J., 2001. A vertebrate fatty acid desaturase with Δ5 and Δ6 activities. Proc. Nat. Acad. Sci. USA 98, 14304–14309.

Higashi, S., Murata, N., 1993. An in vivo study of substrate specificities of acyl-lipid desaturases and acyltransferases in lipid synthesis in *Synechocystis* PCC6803. Plant Physiol 102, 1275–1278.

Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirosawa, M., Sugiura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsuno, A., Muraki, A., Nakazaki, N., Naruo, K., Okumura, S., Shimpo, S., Takeuchi, C., Wada, T., Watanabe, A., Yamada, M., Yasuda, M., Tabata, S., 1996. Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. Strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res 3, 109–136.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, AZ.

López Alonso, D., García-Maroto, F., 2000. Plants as 'chemical factories' for the production of polyunsaturated fatty acids. Biotechnol. Adv 18, 481–497.

Los, D.A., Murata, N., 1998. Structure and expression of fatty acid desaturases. Biochim. Biophys. Acta 1394, 3–15.

Michaelson, L.V., Napier, J.A., Lewis, M., Griffiths, G., Lazarus, C.M., Stobart, A.K., 1998. Functional identification of a fatty acid (Δ5 desaturase gene from *Caenorhabditis elegans*. FEBS Lett 439, 215–218.

Mitchell, A.G., Martin, C.E., 1995. A novel cytochrome b_5-like domain is linked to the carboxyl terminus of the *Saccharomyces cerevisiae* Δ9 fatty acid desaturase. J. Biol. Chemistry 270, 29766–29772.

Murphy, D.J., 1999. Production of novel oils in plants. Curr. Opin. Biotechnol 10, 175–180.

Napier, J.A., Hey, S.J., Lacey, D.J., Shewry, P.R., 1998. Identification of a *Caenorhabditis elegans* Δ6-fatty-acid-desaturase by heterologous expression in *Saccharomyces cerevisiae*. Biochem. J. 330, 611–614.

Napier, J.A., Sayanova, O., Sperling, P., Heinz, E., 1999a. A growing family of cytochrome b_5-domain fusion proteins. Trends Plant Sci 4, 2–4.

Napier, J.A., Michaelson, L.V., Stobart, K., 1999b. Plant desaturases: harvesting the fat of the land. Curr. Opin. Plant Biol 2, 123–127.

Qiu, X., Hong, H., MacKenzie, S.L., 2001. Identification of a Δ4 fatty acid desaturase from *Thraustochytrium* sp. involved in biosynthesis of docosahexaenoic acid by heterologous expression in *Saccharomyces cerevisiae* and *Brassica juncea*. J. Biol. Chemistry 276, 19603–19609.

Saitou, N., Nei, M., 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol 4, 406–425.

Sayanova, O., Beaudoin, F., Libisch, B., Castel, A., Shwery, P.R., Napier, J.A., 2001. Mutagenesis and heterologous expression in yeast of a plant Δ6-fatty acid desaturase. J. Experimental Bot. 52, 1581–1585.

Shanklin, J., Cahoon, E.B., 1998. Desaturation and related modifications of fatty acids. Annu. Rev. Plant Physiol. Plant Mol. Biol 49, 611–641.

Somerville, C., Browse, J., 1996. Dissecting desaturation: plants prove advantageous. Trends Cell Biol 6, 148–153.

Spychalla, J.P., Kinney, A.J., Browse, J., 1997. Identification of an animal ω3 fatty acid desaturase by heterologous expression in *Arabidopsis*. Proc. Nat. Acad. Sci. USA 94, 1142–1147.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The clustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res 24, 4876–4882.