The Journal of Microbiology, April 2008, p. 189-201 Vol. 46, No. 2
DOI 10.1007/s12275-007-0223-3

Fatty Acid Biosynthesis in Eukaryotic Photosynthetic Microalgae: Identification of a Microsomal Delta 12 Desaturase in *Chlamydomonas reinhardtii*

Xiaoyuan Chi[1,2], Xiaowen Zhang[1,2], Xiangyu Guan[3], Ling Ding[1,2], Youxun Li[1,2], Mingqing Wang[3], Hanzhi Lin[1,2], and Song Qin[1]*

[1]*Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, P. R. China*
[2]*Graduate University, Chinese Academy of Sciences, Beijing 100039, P. R. China*
[3]*College of Marine Life Science, Faculty of Life Science and Technology, Ocean University of China, Qingdao 266003, P. R. China*

(Received November 19, 2007 / Accepted January 8, 2008)

Polyunsaturated fatty acids (PUFAs) are important components of infant and adult nutrition because they serve as structural elements of cell membranes. Fatty acid desaturases are responsible for the insertion of double bonds into pre-formed fatty acid chains in reactions that require oxygen and reducing equivalents. In this study, the genome-wide characterization of the fatty acid desaturases from seven eukaryotic photosynthetic microalgae was undertaken according to the conserved histidine-rich motifs and phylogenetic profiles. Analysis of these genomes provided insight into the origin and evolution of the pathway of fatty acid biosynthesis in eukaryotic plants. In addition, the candidate enzyme from *Chlamydomonas reinhardtii* with the highest similarity to the microsomal Δ12 desaturase of *Chlorella vulgaris* was isolated, and its function was verified by heterologous expression in yeast (*Saccharomyces cerevisiae*).

***Keywords*:** fatty acid desaturase, eukaryotic photosynthetic microalgae, *Chlamydomonas reinhardtii*

Algae are a highly diverse group of photosynthetic organisms that are ubiquitous on the earth and critical for maintaining terrestrial and atmospheric conditions (Grossman, 2005). They play a significant role in major biogeochemical processes, primary productivity, and food webs, especially in oligotrophic waters. In recent years, marine microalgae, especially algal oils containing long-chain polyunsaturated fatty acids (LCPUFAs), have received considerable attention due to their production of oils and fatty acids (Tonon *et al*., 2005). These algae are regarded as potential sources for the industrial production of nutritionally important fatty acids, by either large-scale cultivation or transformation of the PUFAs biosynthetic genes to oilseed crops (Meyer *et al*., 2003). Thus, it is not surprising that many genes coding for desaturases with different regioselectivities and from various algae have already been cloned (Sperling *et al*., 2003).

Currently, genomic information is available for over 200 prokaryotes and several eukaryotes, including algae, fungi, plants, animals, and their parasites (Misumi *et al*., 2005). In addition, complete or nearly complete genomic sequences have been recently obtained for the seven eukaryotic photosynthetic microalgae: *Chlamydomonas reinhardtii* (single-celled chlorophyte alga), *Volvox carteri* (multicellular chlorophyte alga), *Phaeodactylum tricornutum* (marine pennate diatom), *Thalassiosira pseudonana* (marine centric diatom), *Cyanidioschyzon merolae* (primitive red alga that lives in sulfate-rich hot springs), and two ecotypes (*O. lucimarinus*; a high light-adapted ecotype, and *O. tauri*; a light-polyvalent ecotype) of the chlorophyte alga *Ostreococcus*. Bioinformatics-based analysis of the various available genomes provides an effective means of gene discovery in focused metabolic situations. In the present study, orthologous genes encoding enzymes of fatty acid desaturation from seven eukaryotic microalgae were identified, and the enzymatic activity of the putative microsomal Δ12 desaturase of *Chlamydomonas reinhardtii* was examined by expression in *Saccharomyces cerevisiae*. The identification of novel desaturases and reconstruction of the pathways involved in unsaturated fatty acid biosynthesis in eukaryotic microalgae will be helpful for the metabolic engineering of fatty acid synthesis in microalgae. Moreover, the characterization of desaturases from microalgae will provide ample candidate genes for the production of the nutritionally important fatty acids in transgenic plants.

Materials and Methods

Computational search for novel fatty acid desaturase genes

The genomes of six eukaryotic microalgae, including *C. reinhardtii*, *V. carteri, O. tauri, O. lucimarinus, P. tricornutum*, and *T. pseudonana*, were downloaded from the JGI database, while the genome of *C. merolae* was available at the website of the *Cyanidioschyzon merolae* Genome Project (http://merolae.biol.s.u-tokyo.ac.jp/). An initial set of fatty acid desaturase genes from *Arabidopsis thaliana* was obtained from the GenBank database (National Center for Biotechnology Information, USA) and used to construct a query protein set. Each protein in this query dataset was used to search the potential novel sequences in the seven eukaryotic micro-

* To whom correspondence should be addressed.
(Tel) 86-532-828-98863; (Fax) 86-532-828-98500
(E-mail) sqin@ms.qdio.ac.cn

Table 1. Candidate genes for the enzymes involved in fatty acid biosynthesis in seven eukaryotic microalgae

Organisms	Locus tag	Proposed function	Amino acids	Sequence similarity (protein)			Identities/ Positives
				Enzyme	Accession No.	Organisms	
Ostreococcus tauri	22819	7	345	ACP-d9	AAD40245	*Brassica juncea*	60%, 75%
	27084	11	361	chld12	AA023565	*Phaeodactylum tricornutum*	55%, 67%
	8220	11	330	chld12	AA023565	*Phaeodactylum tricornutum*	54%, 68%
	9282	16	237	d4	AAX14506	*Thalassiosira pseudonana*	26%, 41%
	19818	unknown	265	d6	AAC15586	*Caenorhabditis elegans*	30%, 45%
	25711	unknown	429	d6AD	CAB94992	*Ceratodon purpureus*	28%, 44%
	8947	14	456	d6	AAW70159	*Ostreococcus tauri*	100%, 100%
	20424	15	491	d5	AAT85663	*Marchantia polymorpha*	45%, 65%
	36188	unknown	487	d5	AAT85663	*Marchantia polymorpha*	26%, 43%
	4076	unknown	291	d7	Q949X0	*Arabidopsis thaliana*	57%, 73%
				d9	AAM12238	*Picea glauca*	55%, 69%
Ostreococcus lucimarinus	15111	7	329	ACP-d9	Q43593	*Olea europaea*	58%, 74%
	18582	11	362	chld12	AA023565	*Phaeodactylum tricornutum*	53%, 56%
	24150	11	387	chld12	AAO23565	*Phaeodactylum tricornutum*	55%, 67%
	94891	unknown	434	d6	AAC15586	*Caenorhabditis elegans*	30%, 45%
	31472	unknown	485	d6AD	CAB94992	*Ceratodon purpureus*	26%, 41%
	3430	14	400	d6	AAW70159	*Ostreococcus tauri*	80%, 89%
	88841	15	491	d5	AAT85663	*Marchantia polymorpha*	46%, 64%
	28687	16	459	d4	AAZ43257	*Thraustochytrium sp. FJN-10*	30%, 46%
	29780	unknown	465	d5	AAT85663	*Marchantia polymorpha*	27%, 46%
	17840	unknown	466	d5	AAT85663	*Marchantia polymorpha*	27%, 46%
	15110	10	498	d8	CAA11857	*Brassica napus*	43%, 60%
	51664	unknown	358	d9	AAM12238	*Picea glauca*	56%, 70%
				d7	Q949X0	*Arabidopsis thaliana*	62%, 76%
Phaeodactylum tricornutum	9316	1	308	ACP-d9	ABS20117	*Pavlova viridis*	59%, 74%
	28797	9	333	d9	AAL99291	*Chanos chanos*	51%, 66%
	25769	11	436	microd12	AAO23564	*Phaeodactylum tricornutum*	100%, 100%
	48423	2	495	chld12	AAO23565	*Phaeodactylum tricornutum*	100%, 100%
	41570	12	435	d15	BAA11397	*Oryza sativa*	33%, 48%
	29488	14	477	d6	AAL92563	*Phaeodactylum tricornutum*	100%, 100%
	50443	unknown	517	d6	AAC15586	*Caenorhabditis elegans*	28%, 43%
	22510	unknown	453	d4	AAQ19605	*Euglena gracilis*	29%, 45%
	22459	15	455	d5	AAT09160	*Nitzschia closterium*	98%, 99%
	46830	15	469	d5	ABP65280	*Phaeodactylum tricornutum*	100%, 100%
Thalassiosira pseudonana	22511	9	338	d9	AAL99291	*Chanos chanos*	50%, 65%
	23798	11	434	microd12	AAO23564	*Phaeodactylum tricornutum*	61%, 75%
	3143	2	473	chld12	AAO23565	*Phaeodactylum tricornutum*	70%, 81%
	41014	12	276	d15	BAA22441	*Zea mays*	35%, 49%
	23391	17	477	d11	AAS75335	*Thalassiosira pseudonana*	100%, 100%
	22544	17	509	d11	AAS75335	*Thalassiosira pseudonana*	60%, 75%
	41113	14	484	d6	AAX14505	*Thalassiosira pseudonana*	100%, 100%
	22976	10	493	d8	AAX14504	*Thalassiosira pseudonana*	100%, 100%
	22804	unknown	515	d8	CAA60621	*Helianthus annuus*	26%, 39%
	22405	unknown	510	d6	AAX22051	*Rhizopus stolonifer*	26%, 42%
	32546	15	476	d5	AAX14502	*Thalassiosira pseudonana*	100%, 100%
	10501	15	482	d5	AAX14502	*Thalassiosira pseudonana*	78%, 87%

Table 1. Continued

Organisms	Locus tag	Proposed function	Amino acids	Sequence similarity (protein)			Identities/ Positives
				Enzyme	Accession No.	Organisms	
Volvox carteri	109049	7	403	ACP-d9	AAF15308	*Persea americana*	63%, 77%
	62507	7	329	ACP-d9	AAL26877	*Bassia scoparia*	60%, 73%
	80845	5	424	chld12	BAA23881	*Chlamydomonas reinhardtii*	86%, 91%
	103047	5	468	chld12	BAA23881	*Chlamydomonas reinhardtii*	33%, 49%
	76038	6	422	d15	BAB78717	*Chlorella vulgaris*	66%, 77%
	66373	11	373	microd12	AAL68983	*Helianthus annuus*	56%, 70%
	92130	13	344	W13	BAE79427	*Chlamydomonas reinhardtii*	68%, 81%
	121243	8	734	d4	AAQ19605	*Euglena gracilis*	31%, 46%
	83090	4	308	d7	Q949X0	*Arabidopsis thaliana*	58%, 72%
	89326	unknown	438	d9	AAM12238	*Picea glauca*	44%, 59%
				d7	Q949X0	*Arabidopsis thaliana*	43%, 61%
Cyanidioschyzon merolae	CMJ201C	9	412	d9	NP_771234	*Bradyrhizobium japonicum*	49%, 65%
	CMM045C	9	476	d9	BAA28834	*Cyanidioschyzon merolae*	100%, 100%
	CMK291C	11	499	chld12	AAO23565	*Phaeodactylum tricornutum*	47%, 60%
Chlamydomonas reinhardtii	205753	7	401	ACP-d9	AAA61558	*Thunbergia alata*	61%, 76%
	117438	7	148	ACP-d9	AAF15308	*Persea americana*	44%, 60%
	56668	5	424	chld12	BAA23881	*Chlamydomonas reinhardtii*	100%, 100%
	174831	5	391	chld12	BAA23881	*Chlamydomonas reinhardtii*	31%, 50%
	135825	11	383	microd12	BAB78716	*Chlorella vulgaris*	59%, 73%
	56237	6	418	d15	BAB78717	*Chlorella vulgaris*	65%, 78%
	182572	13	476	W13	BAE79428	*Chlamydomonas reinhardtii*	100%, 100%
	32523	8	464	d4	AAQ19605	*Euglena gracilis*	31%, 45%
	117883	4	306	d7	Q949X0	*Arabidopsis thaliana*	57%, 73%
	205994	unknown	460	d7	Q949X0	*Arabidopsis thaliana*	50%, 62%
				d9	AAM12238	*Picea glauca*	44%, 59%

* micro, Microsomal; chl, Chloroplastic; d6AD, d6 acetylenase/desaturase; ACP-d9, stearoyl-acyl carrier protein desaturase; 'unknown' means that the function of the gene is uncertain.

algae for which whole genome sequences were available by using the BLASTP and TBLASTN programs, with an *E*-value<1e-10. The searches were iterated until convergence. The distribution of the putative desaturase genes across seven genomes was summarized in Table 1. The other sequences out of the seven eukaryotic microalgae were retrieved from GenBank. The accession numbers of these sequences and the names of corresponding cyanobacteria, eukaryotic algae, higher plants, fungi, and animals were listed in Table 2.

Multiple sequence alignment and phylogenetic analysis

Sequence alignments were generated using the CLUSTAL W program (Thompson *et al*., 1994) and then adjusted manually. Conserved boxes were identified manually using the BioEdit sequence editor. Sequence alignments of genes predicted to be in similar families were used as an input file for the MEGA3 program. A phylogenetic tree was constructed via the neighbor-joining (NJ) method and evaluated with 1,000 rounds of bootstrapping. Three different protein targeting prediction programs were used to estimate the putative subcellular locations of the candidate proteins: IPSORT (http://hypothesiscreator.net/iPSORT) (3), PREDOTAR (http://www.inra.fr/predotar), and TARGETP (http://www.cbs.dtu.dk/services/TargetP).

Strains and growth conditions

The wild-type *C. reinhardtii* 137cc was kindly provided by the Biotechnology Research Institute, Chinese Academy of Agricultural Sciences. Cells were grown in Trisacetate-phosphate (TAP) medium (Harris, 1989), solidified with 1.0% agar. The cultures were incubated at 25°C under 45 μmol photons/m^{-2}/s^{-1} illumination with a 14:10 light:dark cycle. Cells were collected by centrifugation at 2,500×g for 10 min at 22°C. The collected cells were immediately frozen under liquid nitrogen.

Molecular cloning of two Δ12 fatty acid desaturase genes from *C. reinhardtii*

Total RNA was extracted from *C. reinhardtii* using the RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. Specific primers for RT-PCR were designed using the available sequence data from *C. reinhardtii* genome for *C. reinhardtii* CrFad6 (accession number BAA23881) and CrFad2 (locus tag 135825) genes, fad6-1; 5'-TAGGATCCAT

Table 2. List of organisms and desaturase protein sequences analyzed in this study (except for the above sequences from seven eukaryotic microalgal genomes)

Organisms	Accession No.	Label
Arabidopsis thaliana	BAA25180	d9
	Q949X0	d7
	NP_187819	microd12
	AAA92800	chld12
	BAA05514	microd15
	AAB60302	Chld15
	CAA11858	d8
Synechocystis sp. PCC 6803	NP_442430	d9
	NP_441489	d12
	NP_441622	d15
	NP_441824	d6
Caenorhabditis elegans	AAF97550	d9
	AAC15586	d6
	AAC95143	d5
	NP_502560	unknown
Homo sapiens	XP_005719	d9
	AAD20018	d6
	AAF29378	d5
Rattus norvegicus	NP_114029	d9
	BAA75496	d6
	AAG35068	d5
Mortierella alpina	CAB38177	d9
	AAF08684	d12
	AAF08685	d6
Borago officinalis	AAG43277	d8
	AAD01410	d6
Brassica napus	AAF78778	microd12
	AAA50157	chld12
Nicotiana tabacum	BAC01274	chld15
	BAC01273	microd15
Synechococcus sp. PCC 7002	AAF21445	d12
	AAB61352	d15
Chlorella vulgaris	AB075526	microd12
	AB075527	microd15
Thraustochytrium sp. ATCC21685	AAM09688	d4
	AAM09687	d5
Acheta domesticus	AAK25797	d9
Synechococcus elongatus PCC 7942	YP_401578	d9
Caenorhabditis briggsae	CAE58622	unknown
Cyprinus carpio	CAB57858	d9
Drosophila simulans	CAB52475	d9
Gallus gallus	CAA42997	d9
Saccharomyces cerevisiae	AAA34826	d9
Rosa hybrid cultivar	BAA23136	d9
Synechococcus sp. WH 7805	ZP_01124517	unknown
Prochlorococcus marinus NATL2A	YP_291588	unknown
Synechococcus sp. CC9605	YP_382268	unknown
Prochlorococcus marinus pastoris CCMP1986	NP_893499	unknown
Bradyrhizobium japonicum USDA 110	NP_771234	d9
Stigmatella aurantiaca DW4/3-1	ZP_01463016	d9
Myxococcus xanthus DK 1622	YP_634431	d9
Haematococcus pluvialis	ABP57425	ACP-d9
Persea americana	AAF15308	ACP-d9
Linum usitatissimum	CAA07349	ACP-d9
Limnanthes douglasii	AAG28599	ACP-d9
Bassia scoparia	AAL26877	ACP-d9
Olea europaea	Q43593	ACP-d9
Pavlova viridis	ABS20117	ACP-d9
Emericella nidulans	AAG36933	d12
Mucor rouxii	AAD55982	d12
Euglena gracilis	AAQ19605	d4
Thalassiosira pseudonana	AY817156	d4
Isochrysis galbana CCMP1323	AY630574	d4
Nitzschia closterium f. minutissima	AY603475	d5
Marchantia polymorpha	AAT85663	d5
Dictyostelium discoideum	BAA37090	d5
Danio rario	Q9DEX7	d5/d6
Glossomastix chrysoplasta	AAU11444	d6
Arthrospira platensis CG590	ABN11122	d6
Echium pitardii	AAL23581	d6
Mucor circinelloides	BAB69055	d6
Helianthus annuus	CAA60621	d8

* micro, Microsomal; chl, Chloroplastic; ACP-d9, stearoyl-acyl carrier protein desaturase; 'unknown' means that the function of the gene is uncertain.

GGCGTTCGCT-3', and fad6-2; 5'-CCGAATTCTTAGAAGG CGGC-3', fad2-1; 5'-TAGGATCCATGACGGTCACT-3', and fad2-2; 5'-GTGAATTCTCAGCGGTGGTA-3'. The first-strand cDNA was synthesized using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega). PCR reaction of 25 μl contained 0.1 μg of cDNA, 0.01 μm of each primer, 0.02 μm of dNTP and 1 U of Pyrobest DNA polymerase in buffer supplied by the manufacturer of the polymerase (TaKaRa, China). Amplification of the full-length coding sequences of CrFad2 and CrFad6 was performed in Tpersonal Thermocycler (Biometra, Germany) using the following cycle conditions: 94°C, 5 min, 30 cycles (94°C for 1 min, 59°C for 1 min, 72°C for 1.5 min), then extension for 10 min at 72°C. Two expected fragments of 1,152 and 1,275 bp were amplified and then subcloned into the *Bam*HI and *Eco*RI sites of pBluescriptII SK(-) (Stratagene, USA) and sequenced

Table 3. Fatty acid composition of transformed *S. cerevisiae*

Transformant	Percent of total fatty acids					
	14:0	16:0	16:1	18:2	18:1	18:0
p416	2.26	20.55	34.81	–	24.21	18.02
P4CFAD6	1.64	24.78	31.33	–	25.88	16.29
P4CFAD2	1.81	19.05	33.06	4.26	21.90	19.91

* Dashes indicate that the fatty acid was not detected.

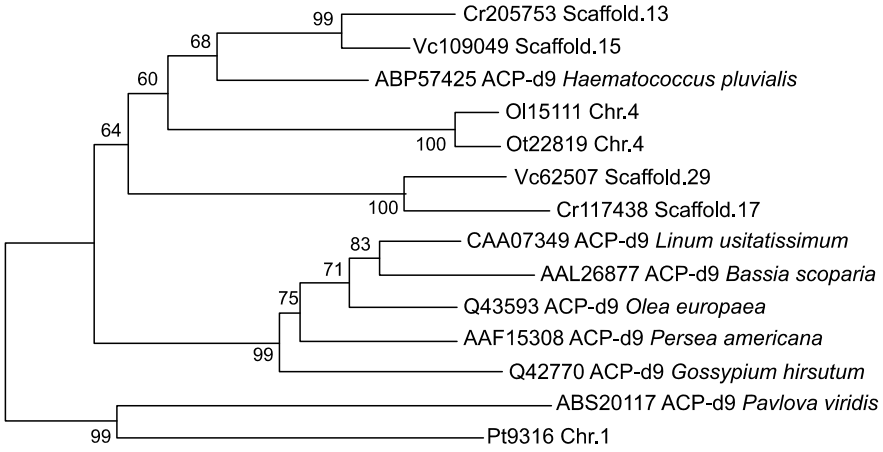

Fig. 1. Neighbor-joining tree based on the deduced amino acid sequences of stearoyl-acyl carrier protein desaturase homologs. Sequences from seven eukaryotic microalgal genomes are shown by their locus tags. The chromosome location or scaffold name for each gene is shown after the gene locus tag and label. Cr, *Chlamydomonas reinhardtii*; Vc, *Volvox carteri*; Ot, *Ostreococcus tauri*; Ol, *Ostreococcus lucimarinus*; Pt, *Phaeodactylum tricornutum*.

by the BioAsia company (Shanghai, China).

Functional characterization of *C. reinhardtii* putative Δ12 desaturases in *S. cerevisiae*

S. cerevisiae K601 (*ade2, his3, leu2, trp1, ura3*) was used as the recipient strain in the transformation experiment. Plasmid p416 is a shuttle vector carrying the URA3 gene for prototrophic selection in *S. cerevisiae* with the constitutive TEF promoter and the CYC1 terminator.

The CrFad2 and CrFad6 sequences in the pBluescriptII SK(-) were excised with *Bam*HI and *Eco*RI and then ligated to plasmid p416, which was digested with the same enzymes to yield the plasmids P4CFAD2 and P4CFAD6. The vectors containing the *C. reinhardtii* sequences were then transformed into *S. cerevisiae* strain K601 using a lithium acetate method. Transformants were selected on minimal medium plates lacking uracil.

Fatty acid analysis

The yeast transformants were cultured in Sc-ura medium at 25°C for 3 days. The yeast cells were harvested and washed with deionized water and then dried by lyophilization. Total lipids were extracted with dichloromethane/methanol (2:1) from dried cells, solidified under nitrogen gas ventilation, and transmethylated with methanol containing 0.5 M KOH-methanol/H_2O (95:5) at 100°C for 2 h. The fatty acid methyl esters (FAMEs) were recovered with n-hexane. FAMEs analysis was carried out using a Finnigan Trace GC-MS equipped with a 30 m×0.25 mm DB-5ms capillary column. Fatty acids were identified by comparing their retention times with those of their FAME standards (Sigma Chemicals Co., USA) separated on the same GC. Measurements were done by using peak height area integrals expressed as a percentage of the total of all integrals.

Results

Phylogenetic analysis of genes from seven eukaryotic microalgal genomes with similarity to fatty acid desaturases

Sixty-seven desaturase genes were predicted and annotated from the seven genomes of eukaryotic microalgae using the BLASTP and TBLASTN programs with the query sequence from *Arabidopsis thaliana*. The candidate pathway genes identified in this study are listed in Table 1. According to the conserved histidine-rich motifs and phylogenetic profiles, these desaturase genes can be divided into 4 major groups: stearoyl-acyl carrier protein (ACP) desaturase group, Δ7/Δ9 desaturase group, Δ12/ω3 desaturase group, and 'front-end' desaturase group (Δ4, Δ5, Δ6, and Δ8 desaturases).

Two major classes of desaturases have been described: soluble and membrane-bound desaturases, both of which are diiron-oxo enzymes (Murphy, 1999). The soluble desaturases were analyzed separately from membrane-bound desaturases because 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). The phylogenetic tree

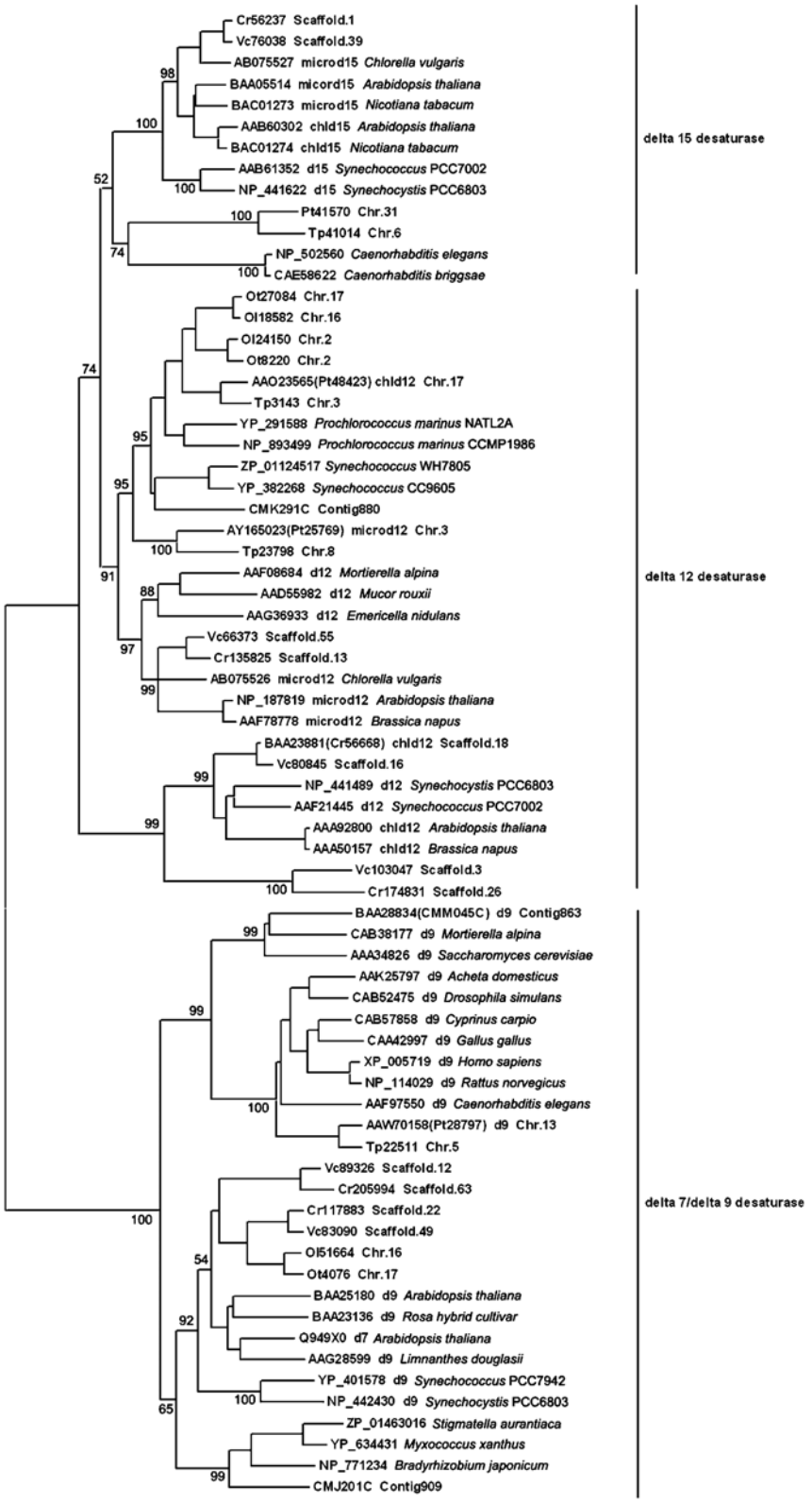

Fig. 2. Neighbor-joining tree based on the deduced amino acid sequences of membrane Δ12, Δ15, and Δ7/Δ9 desaturases. Sequences from seven eukaryotic microalgal genomes are shown by their locus tags. The chromosome location or scaffold (contig) name for each gene is shown after the gene locus tag and label. Desaturase genes with functional characterization from seven eukaryotic microalgae are represented by their accession numbers with their locus tags in parentheses. Cr, *Chlamydomonas reinhardtii*; Vc, *Volvox carteri*; Ot, *Ostreococcus tauri*; Ol, *Ostreococcus lucimarinus*; Pt, *Phaeodactylum tricornutum*; Tp, *Thalassiosira pseudonana*; Cm, *Cyanidioschyzon merolae*.

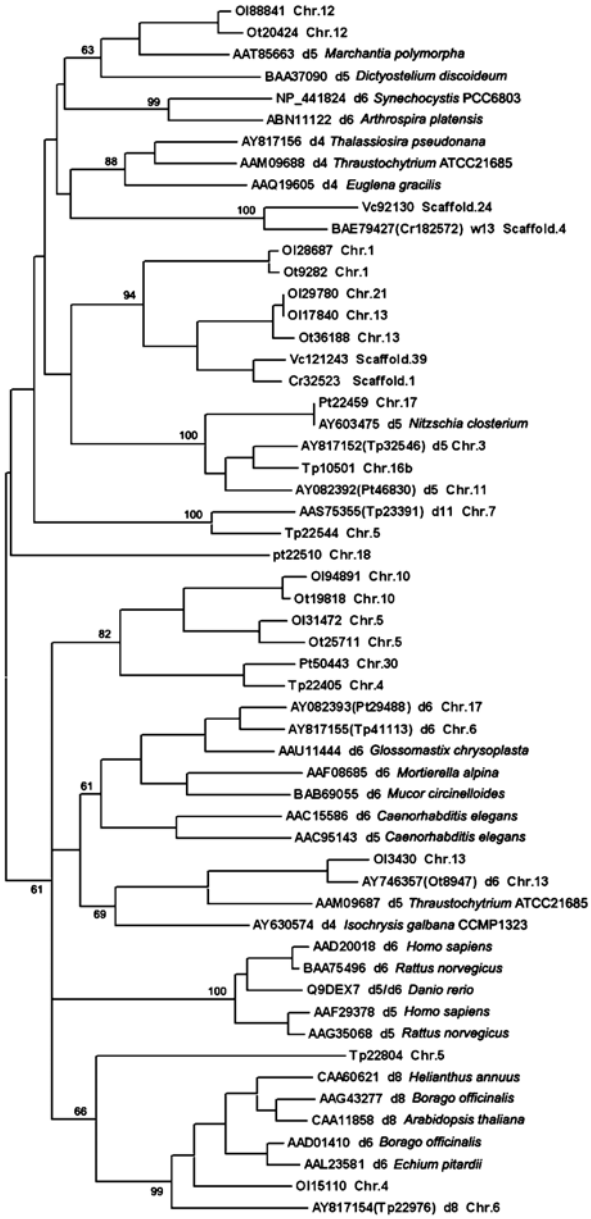

Fig. 3. Neighbor-joining tree based on the deduced amino acid sequences of front-end desaturase homologs. Sequences from seven eukaryotic microalgal genomes are shown by their locus tags. The chromosome location or scaffold name for each gene is shown after the gene locus tag and label. Desaturase genes with functional characterization from seven eukaryotic microalgae are represented by their accession numbers with their locus tags in parentheses. Cr, *Chlamydomonas reinhardtii*; Vc, *Volvox carteri*; Ot, *Ostreococcus tauri*; Ol, *Ostreococcus lucimarinus*; Pt, *Phaeodactylum tricornutum*; Tp, *Thalassiosira pseudonana*; Cm, *Cyanidioschyzon merolae*.

shows that the prospective stearoyl-ACP desaturases from five green algae were grouped with those from higher plants and set apart from the genes of Chrysophyceae and Bacillariophyceae, which reflects a fundamentally different evolutionary history between Plantae and Chromalveolates (Fig. 1). It is intriguing that the gene encoding stearoyl-ACP desaturase was absent in *T. pseudonana* and *C. merolae*, but was found in *P. tricornutum*. It remains to be determined whether the gene encoding the stearoyl-ACP desaturase was just missed in the genomic analysis or a novel (or highly diverged) substituted enzyme appeared. Moreover, phylogenetic analysis suggests that stearoyl-ACP desaturases in green algae and higher plants arose by independent gene duplication events.

As shown in the phylogenetic tree, all of the membrane-bound desaturases fell into two distinct subfamilies: the Δ7/Δ9 desaturase subfamily and Δ12/ω3 desaturase subfamily (Fig. 2). The Δ7/Δ9 desaturase subfamily was clustered into two subgroups. The putative Δ9 desaturases from green algae were grouped together with those from cyanobacteria and

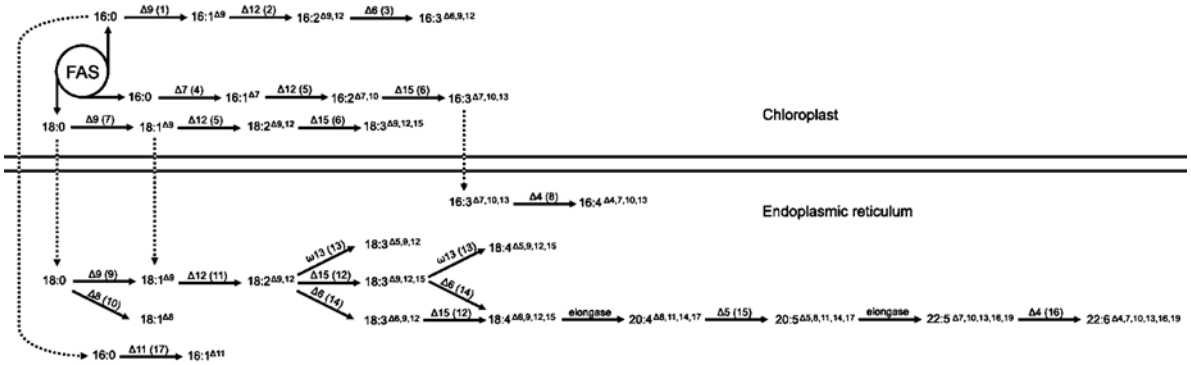

Fig. 4. The putative pathway of fatty acid biosynthesis in eukaryotic microalgal cells. Fatty acid modifications are indicated by arrows, and possible exchange reactions are indicated by dashed lines. Numbers above the arrowheads indicate the positions at which a double bond is introduced, and the numbers in parentheses serve to cross reference the specific reactions with the respective enzymes and gene locus tags summarized in Table 1. Fatty acids are identified as "carbons:double bonds." FAS, Fatty acid synthase.

```
  -20       -10         0         10        20        30        40        50        60        70        80
TTACGTATTGCAAGTTGTAACAAAATGACGGTCACTCGGCGCAAGGGCGTCAACATCCAGGCCGATGCGACCGATTCGGCTGGGGAGAAGCAGCGGTACCCTGCGGCTCC
                          M  T  V  T  R  R  K  G  V  N  I  Q  A  D  A  T  D  S  A  G  E  K  Q  R  Y  P  A  A  P

  90        100       110       120       130       140       150       160       170       180       190
GCCGACATTCTCCCTGGGAGACATCCGCAAGGCTATTCCTGCGCACTGCTTCGAGAAATCCGCACTGCGCAGCTTTGCTCACCTGGCCGTTGATGTGACCGTCTGCGCCT
  P  T  F  S  L  G  D  I  R  K  A  I  P  A  H  C  F  E  K  S  A  L  R  S  F  A  H  L  A  V  D  V  T  V  C  A

 200       210       220       230       240       250       260       270       280       290       300
GGCTGTGGTACGGGTCGACGTTCATCGACCACCCAGCCGTGCCCCGCTACCTGGCCTGGTTCGTGCTGTGGCCTCTGTACTGGTTCTGGCAGGGCGCCTTCATGACGGGC
  W  L  W  Y  G  S  T  F  I  D  H  P  A  V  P  R  Y  L  A  W  F  V  L  W  P  L  Y  W  F  W  Q  G  A  F  M  T  G

 310       320       330       340       350       360       370       380       390       400       410
ATCTGGGTCATTGCGCACGAGTGCGGCCACGGCGCCTTCTCCAACAGCGAGGCGCTCAACGACGGCGTGGGCCTGGTGATGCACTCGCTGCTGCTGGTGCCCTATTACAG
  I  W  V  I  A  H  E  C  G  H  G  A  F  S  N  S  E  A  L  N  D  G  V  G  L  V  M  H  S  L  L  L  V  P  Y  Y  S

 420       430       440       450       460       470       480       490       500       510       520
CTGGAAGCACTCGCACCGACGCCACCACCAGAACACGGGCAGCACGGCCAAGGACGAGGTGTTTGTGCCGGCAGTCAAGCCCGCGGGCACTAAGGCTCCCTGGTACCACC
  W  K  H  S  H  R  R  H  H  Q  N  T  G  S  T  A  K  D  E  V  F  V  P  A  V  K  P  A  G  T  K  A  P  W  Y  H

 530       540       550       560       570       580       590       600       610       620       630
GCAACCCCGTGTACCGCCTGGGCCACATCCTGTTCCAGCAGCTGCTGGGCTGGCCGCTGTACCTGCTGTTCAACGTGTCGGGCCACGAATACCCGCGCTGGGCCAACCAT
  R  N  P  V  Y  R  L  G  H  I  L  F  Q  Q  L  L  G  W  P  L  Y  L  L  F  N  V  S  G  H  E  Y  P  R  W  A  N  H

 640       650       660       670       680       690       700       710       720       730       740
TTCGACCCCTTCTCGCCCATCTTCACCAAGCGCGAGCGCATTGAGGTGCTGGTCTCTGATATTGCGCTGGCGGTGGTCGTGGCCGGGCTGGCCGCCATCAGCCGCACCTG
  F  D  P  F  S  P  I  F  T  K  R  E  R  I  E  V  L  V  S  D  I  A  L  A  V  V  V  A  G  L  A  A  I  S  R  T  W

 750       760       770       780       790       800       810       820       830       840       850
GGGCTTCATGTTCCTACTTAAGACGTACCTCATCCCCTACCTGGTGGTCAACCACTGGCTGGTGATGATCACCTTCCTGCAGCACACGCACCCCAAGCTGCCGCACTACG
  G  F  M  F  L  L  K  T  Y  L  I  P  Y  L  V  V  N  H  W  L  V  M  I  T  F  L  Q  H  T  H  P  K  L  P  H  Y

 860       870       880       890       900       910       920       930       940       950       960
GCGACGGCGAGTGGGACTGGCTGCGCGGCGCCATGGCCACCGTGGACCGCTCCTACGGCGTGCTGGACCACGTGTTCCACCACATCGCCGACACGCACGTTGCGCACCAC
  G  D  G  E  W  D  W  L  R  G  A  M  A  T  V  D  R  S  Y  G  V  L  D  H  V  F  H  H  I  A  D  T  H  V  A  H  H

 970       980       990       1000      1010      1020      1030      1040      1050      1060      1070
CTGTTCTCCTACATGCCGCACTATCACGCGGAGGAGGCCACCGAGGCCATCAAGAAGGTGTTGGGTGACTACTACGCCTACGACAGCCGCAACGTGTTCCGCGCGCTGTG
  L  F  S  Y  M  P  H  Y  H  A  E  E  A  T  E  A  I  K  K  V  L  G  D  Y  Y  A  Y  D  S  R  N  V  F  R  A  L  W

 1080      1090      1100      1110      1120      1130      1140      1150      1160      1170
GGATGAGGTGGGCGGCTGCGCGGTGGTGGCGCCGGACACCAACGGCCCGGAGCAGGTGTACTGGTACCACCGCTGAGGCGGGGGAGGAGCAGGAGAAGGAG
  D  E  V  G  G  C  A  V  V  A  P  D  T  N  G  P  E  Q  V  Y  W  Y  H  R  *
```

Fig. 5. Nucleotide and deduced amino acid sequence of the cDNA for CrFad2. The amino acid sequence of the putative coding region is shown by one-letter code. The stop codon is indicated by an asterisk.

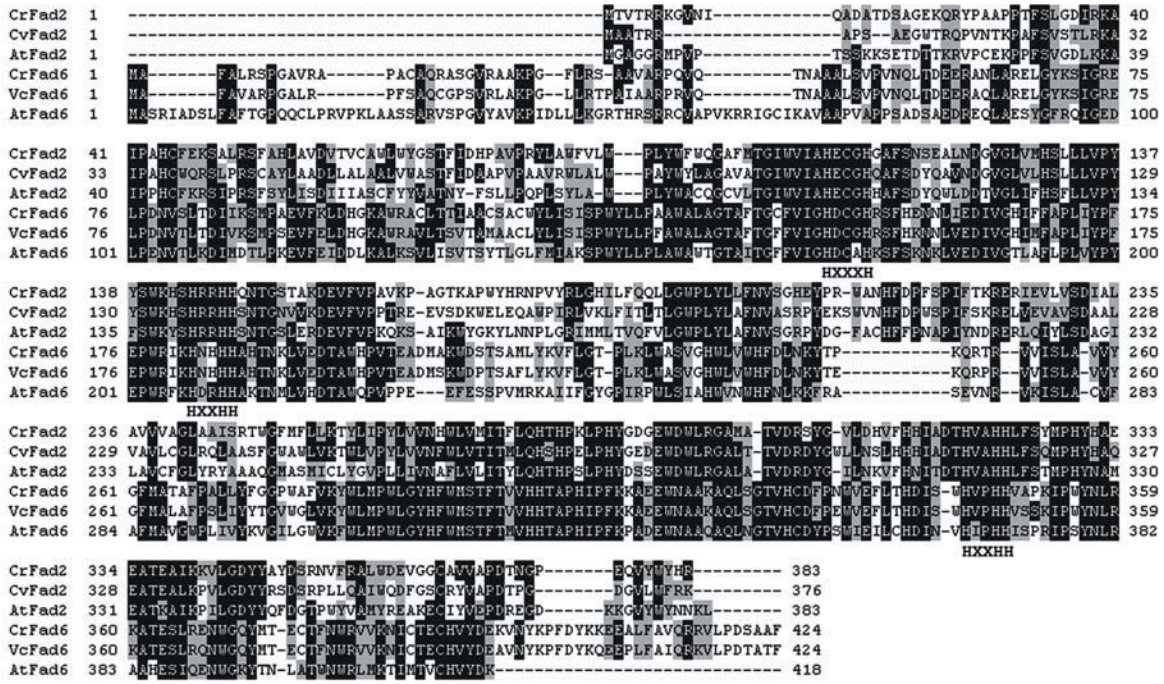

Fig. 6. Amino acid sequences of CrFAD6 and CrFAD2 in comparison with the plastidial and microsomal homologs from other plants. Three conserved histidine-rich motifs are indicated by subscripts. Accession numbers or locus tags for the sequences were as follows: *Chlamydomonas reinhardtii* CrFad2 (Cr135825) and CrFad6 (BAA23881), *Chlorella vulgaris* CvFad2 (AB075526), *Arabidopsis thaliana* AtFad2 (NP_187819) and AtFad6 (AAA92800), *Volvox carteri* VcFad6 (Vc80845). The conserved amino acids are shaded.

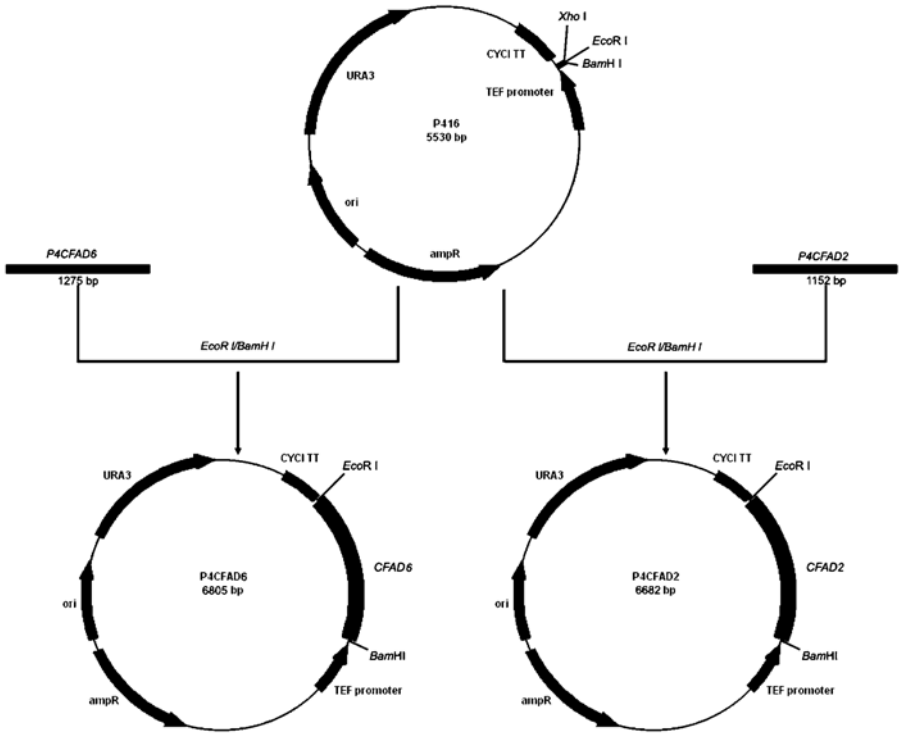

Fig. 7. Construction of yeast expression vector P4CFAD2/P4CFAD6. In addition to CrFad2 or CrFad6, the vector contains the plasmid origin (ori), the URA3 gene marker, the TEF promoter, and the CYC1 terminator. CrFad2 and CrFad3 were subcloned into p416 to generate plasmids designated as P4CFAD2 and P4CFAD6, respectively.

higher plants, while the Δ9-homologous genes from the diatom *P. tricornutum* and *T. pseudonana* fell into the group of animals. It is worth noting that one Δ9 desaturase from *C. merolae* was fused with the cytochrome b_5 domain on its carboxyl terminus and grouped with desaturases from *S. cerevisiae*. The other Δ9 desaturase from *C. merolae* formed a group with the desaturase genes from protobacteria and set apart from the groups of higher plants and green algae. Therefore, the Δ9 desaturase may arise by independent gene duplication events in animal/fungi and plant/cyanobacteria branches.

In the Δ12/ω3 desaturase subfamily, the chloroplastic Δ12 desaturases from *C. reinhardtii* and *V. carteri* were situated along with Δ12 desaturases from cyanobacteria and chloroplastic Δ12 desaturases of higher plants at the basal position of the tree, leading to cluster I. In cluster II, the microsomal Δ12 desaturase genes from *C. reinhardtii* and *V. carteri* clustered together with genes from fungi and higher plants and were separated from those of marine cyanobacteria, diatoms, rhodophytes and prasinophyceae. In cluster III, the ω3 desaturases from cyanobacteria were placed in a basal position, grouped with both microsomal and chloroplastic ω3 desaturases from higher plants and eukaryotic algae and set apart from enzymes from *P. tricornutum*, *T. pseudonana,* and *Caenorhabditis elegans*. Phylogenetic analysis suggests that ω3 desaturases arose by independent gene duplication events from a Δ12 desaturase ancestor in the diatom/nematode and plant/cyanobacterial branches, and the cyanobacterial Δ12 desaturase was identified as the origin of the plant ω3 desaturases, including both chloroplast and endoplasmic reticulum (ER) isozymes. The phylogenetic tree also suggests that ω3 desaturases from eukaryotic microalgae and higher plants arose by independent gene duplication events.

The 'front-end' desaturases (Δ4, Δ5, Δ6, and Δ8 desaturases) formed a separate clade (Fig. 3), and their phylogeny is complicated. It has been speculated that front-end desaturases may have the same origin, but their precise lineages are still unclear. These desaturases fell into two separate subgroups. Cluster I of subgroup I was composed of Δ6 desaturases from cyanobacteria, Δ5 desaturases from fungi, green algae and *Dictyostelium*, Δ4 desaturases from *Thraustochytrium T. pseudonana* and *E. gracilis*. Cluster II was integrated by Δ5 desaturases from *Bacillariophyta* and Δ4 desaturase from green algae. One cluster in subgroup II was composed of desaturases from *O. tauri, O. lucimarinus, P. tricornutum*, and *T. pseudonana* with uncertain functions. Another cluster in subgroup II was integrated by Δ6 desaturases from fungi, moss, algae, Δ5 and Δ6 desaturases from the nematode *Caenorhabditis*, Δ4 desaturase of *Isochrysis galbana* and Δ5 desaturase from *Thraustochytrium*. The third cluster in subgroup II included Δ5 and Δ6 desaturases from vertebrates; and the last cluster was constituted by Δ6 desaturases from higher plants and Δ8-sphingolipid desaturases from higher plants and algae.

Functional characterization of *Chlamydomonas reinhardtii* Δ12 desaturase-related proteins by expression in yeast *S. cerevisiae*

Phylogenetic analysis of the genome sequence of *C. reinhardtii* revealed the presence of one sequence showing sig-

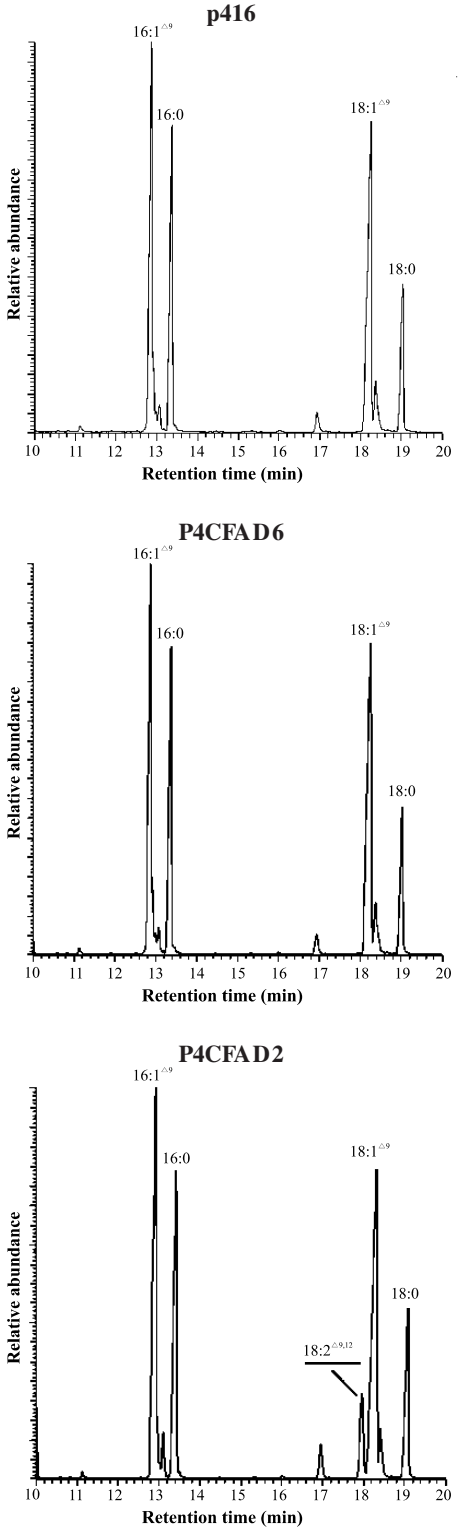

Fig. 8. Fatty acid profiles of transgenic yeasts expressing microsomal and plastidial desaturases. The K601 yeast strain was transformed with the indicated plasmids. The transformants were grown for 72 h at 25°C, and FAMEs from whole cells were prepared and analyzed by gas chromatography (GC) as indicated in 'Materials and Methods'. New fatty acids are underlined. The experiment was repeated twice and the results of a representative experiment were shown.

nificant similarity to functionally characterized microsomal Δ12 desaturases (59%). We re-examined this putative desaturase sequence (locus tag 135825, designated as CrFad2) based on cDNA characterization and genome analysis. Comparison of the full-length CrFad2 cDNA with the genomic sequence demonstrated that nine introns are present in the gene. The CrFad2 ORF gives a predicted protein of 383 amino acids (Fig. 5). Three histidine boxes were found in all known microsomal Δ12 desaturases, forming part of the diiron center where oxygen activation and substrate oxidation occured, and they were conserved in the CrFad2 amino acid sequence (Fig. 6).

To characterize the function of this putative Δ12 desaturase, full-length cDNA of CrFad2 was cloned into the yeast expression vector p416 to produce the vector P4CFAD2 (Fig. 7). A gene for chloroplast Δ6 desaturase (GenBank accession number BAA23881, designated as CrFad6), which catalyzed the desaturation of monoenoic acids to dienoic acids in the chloroplasts of *C. reinhardtii* (Sato *et al*., 1997), was used to yield the vector P4CFAD6 (Fig. 7). Transformants containing p416 and P4CFAD6 were used for control and comparison, respectively. After 72 h of incubation, total lipids were extracted from the transformants and subjiected to GC analysis.

The transformants with the empty vector p416 showed a rather simple fatty acid profile with 16:0, $16:1^{\Delta 9}$, $18:1^{\Delta 9}$, and 18:0 as the major fatty acids (Fig. 8). The fatty acid profile of transformants with P4CFAD6 did not change. Yeast is known to be the model of choice for the functional characterization of microsomal FADs because it contains the short electron transport system required by these desaturases (i.e., cytochrome b5 and NADH-cytochrome b5 reductase) (Domergue *et al*., 2003). Desaturases of plastidial origin usually require ferredoxin and NADPH-ferredoxin reductase for their short electron transport system, which may make chloroplastic desaturase inactive in *S. cerevisiae*.

In contrast, a novel peak corresponding to dienoic fatty acids C18:2 appeared in the fatty acid profile of the transformants containing P4CFAD2, and the percentage was 4.26%. The reduced amount of C18:1 in the P4CFAD2 transformant was explained by the conversion of C18:1 into C18:2, which should be catalyzed by a microsomal Δ12 fatty acid desaturase encoded by CrFad2 from *C. reinhardtii*.

Discussion

In the present study, the genomes of seven eukaryotic microalgae were analyzed using bioinformatic methods, and 67 desaturase genes were found. A fatty acid biosynthesis pathway from palmitic acid (16:0) to docosahexaenoic acid ($C22:6\Delta^{4,7,10,13,16,19}$, DHA) in algae was proposed as shown in Fig. 4.

It is well known that higher plants and green, red and glaucophyte algae are derived from a primary endosymbiotic event in which a non-photosynthetic eukaryote acquired a chloroplast by engulfing (or being invaded by) a prokaryotic cyanobacterium. In contrast, dominant bloom-forming eukaryotic phytoplankton, such as diatoms and haptophytes, found in the ocean are derived by secondary endosymbiosis, whereby a non-photosynthetic eukaryote acquired a chloroplast by engulfing a photosynthetic eukaryote, probably a red algal endosymbiont. Each endosymbiotic event led to new combinations of genes derived from the hosts and endosymbionts (Falkowski *et al*., 2004).

Four of seven phtosynthetic algae are green algae. *C. reinhardtii* and *V. carteri* belong to Chlorophyte, whereas *O. tauri* and *O. lucimarinus* belong to Prasinophyceae, an early-diverging class within the green plant lineage (Chretiennot-Dinet *et al*., 1995). *P. tricornutum* and *T. pseudonana* are unicellular diatoms belonging to Stramenopiles and are thought to have arisen from a secondary endosymbiosis between a red alga (Rhodophyta) and a heterotrophic flagellate (related to the Oomycetes) around 300 million years ago (Gibbs, 1981; Delwiche and Palmer, 1997). *C. merolae*, a unicellular rhodophyte, is one of the most primitive red alga and probably diverged from near the root of the red lineage (Sato and Moriyama, 2007).

C. reinhardtii and *V. carteri*

C. reinhardtii is a unicellular, soil-dwelling green alga whose lineage diverged from land plants over one billion years ago (Merchant *et al*., 2007). The genome of *C. reinhardtii* is approximately 110 Mb in size, with nearly 95 Mb of the sequence completed; however, the sequence information is still dispersed over approximately 3,000 individual scaffolds (Grossman *et al*., 2005). *V. carteri*, the 'fierce roller', is a multicellular chlorophyte alga that is closely related to the single-celled *C. reinhardtii*. The genome of *V. carteri* is estimated to be 140 Mb in size. The main fatty acid compositions of *C. reinhardtii* strain 137c are C16:0, $C16:1\Delta^{7}$, $C16:4\Delta^{4,7,10,13}$, $C18:1\Delta^{9}$, $C18:2\Delta^{9,12}$, $C18:3\Delta^{9,12,15}$, $C18:3\Delta^{5,9,12}$ (PA) and $C18:3\Delta^{5,9,12,15}$ (CA) (Giroud *et al*., 1988). Two Δ5-unsaturated bis-methylene-interrupted fatty acids (UBIFAs), which are widely distributed among the gymnosperms, are present in *C. reinhardtii* (Giroud and Eichenberger, 1989). *V. carteri* also contains PA and CA, but no C_{20} PUFAs, as in the case of *C. reinhardtii* (Kajikawa *et al*., 2006). Thus, the types of desaturases and pathway of fatty acid biosynthesis in these two green algae are almost the same. As in the model plant *Arabidopsis*, there are two interconnected pathways of fatty acid biosynthesis in *C. reinhardtii* and *V. carteri*. The so-called "eukaryotic" pathway is located in the ER and involved in the synthesis of $18:3\Delta^{5,9,12}$ and $18:4\Delta^{5,9,12,15}$. In the chloroplast, the "prokaryotic" pathway is responsible for the synthesis of $16:3\Delta^{7,10,13}$ and $C18:3\Delta^{9,12,15}$. Additionally, the data issued from the genome of *C. reinhardtii* were used to amplify a gene encoding a putative Δ12 desaturase involved in polyunsaturated fatty acid biosynthesis. Heterologous expression in *S. cerevisiae* revealed that it was a microsomal Δ12 fatty acid desaturase.

O. tauri and *O. lucimarinus*

Ostreococcus have a strikingly simple cellular organization, with a single chloroplast and mitochondrion, but no cell wall or flagella (Chretiennot-Dinet *et al*., 1995). It is reported to be a globally abundant, single-celled alga thriving in the upper (illuminated) water column of the oceans (Derelle *et al*., 2002). Three different ecotypes or potential species have been defined based on their adaptation to light intensity. The genomes of two of these strains, *O. lucimarinus* and *O.*

tauri, have been sequenced. *O. lucimarinus* is a surface-isolated strain and adapted to high light intensities. *O. tauri* was isolated from a coastal lagoon and considered to be light-polyvalent (Palenika *et al*., 2007). *O. lucimarinus* has a nuclear genome size of 13.2 Mb found in 21 chromosomes, while that in *O. tauri* is 12.6 Mb found in 20 chromosomes (Derelle *et al*., 2006).

O. tauri contains polyunsaturated fatty acids, such as arachidonic acid (C20:4$\Delta^{5,8,11,14}$), eicosapentaenoic acid (C20:5$\Delta^{5,8,11,14,17}$) and docosahexaenoic acid (C22:6$\Delta^{4,7,10,13,16,19}$). Although little is known about the fatty acid profile of *O. lucimarinus*, our results suggest that the pathway of fatty acid metabolism of this alga is similar to that of *O. tauri*. Few desaturase genes have been functionally characterized in these two algae, and Domergue cloned the first acyl-CoA Δ6-desaturase from *O. tauri* (Domergue *et al*., 2005), which showed very high desaturation activity when expressed in *S. cerevisiae*. Co-expression of this desaturase with an acyl-CoA elongase and the lipid-linked Δ5-desaturase also confirmed that this enzyme was an acyl-CoA Δ6-desaturase. The pathway of fatty acid metabolism for these two algae will not be clear until functions of the putative desaturases are characterized.

C. merolae

The red alga *C. merolae* is an ultra-small (1.5 mm in diameter) unicellular organism that lives in the extreme environment of acidic hot springs (pH 1.5, 45°C; De Luca *et al*., 1978). It is thought to be one of the most primitive photosynthetic eukaryotes (Seckbach, 1994; Nozaki *et al*., 2003; Yoon *et al*., 2006). The complete sequenced genome of *C. merolae* is approximately 16.5 Mb, with 5,331 genes packed into 20 chromosomes (Misumi *et al*., 2005).

C. merolae possesses only saturated and monounsaturated acids. Palmitic (16:0) and linoleic (18:2) acids are the major fatty acids found in *C. merolae* (Sato and Moriyama, 2007). Only three desaturase genes were found in this red alga, two for Δ9 desaturase and one for Δ12 desaturase. The limited amount of desaturase genes in this alga may be due to their specialized environmental niche. Phylogenetic analysis reveals that the two Δ9 desaturases from *C. merolae* diverged from both cyanobacteria DesC and stearoyl-ACP desaturase, showing no direct relationship with Δ9 desaturases from plants or cyanobacteria (Sato and Moriyama, 2007). Moreover, the Δ12 desaturase of *C. merola,* which was grouped with enzymes from marine cyanobacteria, diatoms, and prasinophyceae, diverged from the microsomal Δ12 desaturases from green algae, fungi and higher plants. These results may suggest that the Δ12 desaturases from *C. merolae*, prasinophyceae and diatoms are probably acquired by horizontal gene transfer from the marine cyanobacteria *Synechococcus* and *Prochlorococcus*.

P. tricornutum and *T. pseudonana*

Diatoms are a ubiquitous class of microalgae of extreme importance for global primary productivity and for the biogeochemical cycling of minerals, such as silica (Scala *et al*., 2002). They may contribute as much as 25% of the total primary production on earth (Scala and Bowler, 2001). *P. tricornutum*, a silica-less diatom with a small genome size of about 20 Mb, is mainly known as a potential source for the industrial production of eicosapentaenoic acid (Molina *et al*., 1996). The EPA content of this organism is about 30% (Domergue *et al*., 2003). *T. pseudonana* is a diatom that can produce EPA and DHA, the levels of which are about 17 and 5%, respectively, in the exponential growth phase (Tonon *et al*., 2002). *T. pseudonana* consists of 24 chromosomes, and the genome size is approximately 34 Mb (Armbrust *et al*., 2004; Grossman *et al*., 2005).

It is speculated that two fatty acid biosynthetic pathways co-exist in *P. tricornutum*. The prokaryotic pathway in the plastid leads to the synthesis of 16:3$\Delta^{6,9,12}$, whereas the eukaryotic pathway in the ER contributes to the synthesis of EPA (Domergue *et al*., 2003). The results presented in this study demonstrate that the pathway of *P. tricornutum* is similar to that of *T. pseudonana*. Two Δ9 desaturases were found in *P. tricornutum*, a soluble stearoyl-ACP desaturase and a membrane-bound Δ9-acyl-lipid desaturase, while just one membrane-bound Δ9-acyl-lipid desaturase was found in *T. pseudonana*. The definite function and localization of these Δ9 genes remain unclear. Although the gene corresponding to a plastidial Δ6 desaturase was not detected in this study, the candidate gene for a ω3 desaturase was found, which will lead to a better understanding of the fatty acid metabolism in diatoms.

To summarize, polyunsaturated fatty acids (PUFA) play important roles in adult and infant nutrition because they serve as precursors of eicosanoids, including prostaglandins and leukotrienes. They are also necessary for membrane structure and function, the regulation of cholesterol metabolism and infant brain development (Horrobin, 1992). The isolation and identification of the desaturase genes from these algae should contribute to the development of transgenic oil plants, such as rapeseed, that are capable of producing UBIFAs and VLCPUFAs, leading to the production of low-cost commercial-scale UBIFA and VLCPUFAs for the improvement of human health.

Acknowledgements

This work was supported by funds from the National Natural Science Foundation of China (30670165), Hi-Tech Research and Development Program (2006AA090303) of China, and the CAS/SAFEA International Partnership Program for Creative Research Teams (Research and Applications of Marine Functional Genomics).

References

Armbrust, E.V., J.A. Berges, C. Bowler, B.R. Green, D. Martinez, N.H. Putnam, S. Zhou, A.E. Allen, K.E. Apt, M. Bechner, M.A. Brzezinski, B.K. Chaal, A. Chiovitti, A.K. Davis, M.S. Demarest, J.C. Detter, T. Glavina, D. Goodstein, M.Z. Hadi, U. Hellsten, *et al*. 2004. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism. *Science* 306, 79-86.

Chretiennot-Dinet, M.J., C. Courties, A. Vaquer, J. Neveux, H. Claustre, J. Lautier, and M.C. Machado. 1995. A new marine picoeucaryote: *Ostreococcus tauri* gen. et sp. nov. (Chlorophyta, Prasinophyceae). *Phycologia* 34, 285-292.

De Luca, P., R. Taddei, and L. Varano. 1978. *Cyanidioschyzon mer-*

olae: a new alga of thermal acidic environments. *Webbia* 33, 37-44.

Delwiche, C.F. and J.D. Palmer. 1997. The origin of plastids and their spread via secondary symbiosis, p. 53-86. *In* D. Bhattacharya (ed.), Origins of Algae and their plastids. Springer-Verlag, Vienna, Austria.

Derelle, E., C. Ferraz, P. Lagoda, S. Eychenié, R. Cooke, F. Regad, X. Sabau, C. Courties, M. Delseny, J. Demaille, A. Picard, and H. Moreau. 2002. DNA libraries for sequencing the genome of *Ostreococcus tauri* (Chlorophyta, Prasinophyceae): the smallest free-living eukaryotic cell. *J. Phycol.* 38, 1150-1156.

Derelle, E., C. Ferraz, S. Rombauts, P. Rouze, A.Z. Worden, S. Robbens, F. Partensky, S. Degroeve, S. Echeynie, R. Cooke, Y. Saeys, J. Wuyts, K. Jabbari, C. Bowler, O. Panaud, B. Piégu, S.G. Ball, J.-P. Ral, F.-Y. Bouget, G. Piganeau, *et al*. 2006. Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. *Proc. Natl. Acad. Sci. USA* 103, 11647-11652.

Domergue, F., A. Abbadi, U. Zähringer, H. Moreau, and E. Heinz. 2005. *In vivo* characterization of the first acyl-CoA Δ^6-desaturase from a member of the plant kingdom, the microalga *Ostreococcus tauri*. *Biochem. J.* 389, 483-490.

Domergue, F., P. Spiekermann, J. Lerchl, C. Beckmann, O. Kilian, P.G. Kroth, W. Boland, U. Zähringer, and E. Heinz. 2003. New insight into *Phaeodactylum tricornutum* fatty acid metabolism. cloning and functional characterization of plastidial and microsomal △12-fatty acid desaturases. *Plant Physiology* 131, 1648-1660.

Falkowski, P.G., M.E. Katz, A.H. Knoll, A. Quigg, J.A. Raven, O. Schofield, and F.J. Taylor. 2004. The evolution of modern eukaryotic phytoplankton. *Science* 305, 354-360.

Harris, E.H. 1989. The Chlamydomonas sourcebook: a comprehensive guide to biology and laboratory use, p. 31-52. Academic Press, San Diego, USA.

Horrobin, D.F. 1992. Nutritional and medical importance of gamma-linoleic acid. *Prog. Lipid Res.* 31, 163-194.

Gibbs, S.P. 1981. The chloroplasts of some algal groups may have evolved from endosymbiotic eukaryotic algae. *Ann. NY Acad. Sci.* 361, 193-208.

Giroud, C. and W. Eichenberger. 1989. Lipids of *Chlamydomonas reinhardtii*. Incorporation of [^{14}C]acetate, [^{14}C]palmitate and [^{14}C]oleate into different lipids and evidence for lipid-linked desauration of fatty acids. *Plant Cell Physiol.* 30, 121-128.

Giroud, C., A. Gerber, and W. Eichenberger. 1988. Lipids of *Chlamydomonas reinhardtii*. Analysis of molecular species and intracellular site(s) of biosynthesis. *Plant Cell Physiol*. 29, 587-595.

Grima, E.M., A.R. Medina, A.G. Giménez, and M.J.I. González. 1996. Gram-scale purification of eicosapentaenoic acid (EPA, 20: 5n-3) from wet *Phaeodactylum tricornutum* UTEX 640 biomass. *J. Appl. Phycol.* 8, 359-367.

Grossman, A.R. 2005. Paths toward algal genomics plant. *Physiology* 137, 410-427.

Kajikawa, M., K.T. Yamato, Y. Kohzu, S. Shoji, K. Matsui, Y. Tanaka, Y. Sakai, and H. Fukuzawa. 2006. A front-end desaturase from *Chlamydomonas reinhardtii* produces pinolenic and coniferonic acids by ω13 desaturation in methylotrophic yeast and tobacco. *Plant Cell Physiol* 47, 64-73.

Merchant, S.S., S.E. Prochnik, O. Vallon, E.H. Harris, S.J. Karpowicz, G.B. Witman, A. Terry, A. Salamov, L.K. Fritz-Laylin, L. Maréchal-Drouard, W.F. Marshall, L.H. Qu, D.R. Nelson, A.A. Sanderfoot, M.H. Spalding, V.V. Kapitonov, Q. Ren, P. Ferris, E. Lindquist, H. Shapiro, *et al*. 2007. The Chlamydomonas genome reveals the evolution of key animal and plant functions. *Science* 318, 245-250.

Meyer, A., P. Cirpus, C. Ott, R. Schlecker, U. Zähringer, and E. Heinz. 2003. Biosynthesis of docosahexaenoic acid in *Euglena gracilis*: biochemical and molecular evidence for the involvement of a Δ4-fatty acyl group desaturase. *Biochemistry* 42, 9779-9788.

Misumi, O., M. matsuzaki, H. Nozaki, S. Miyagishima, T. Mori, K. Nishida, F. Yagisawa, Y. Yoshida, H. Kuroiwa, and T. Kuroiwa. 2005. *Cyanidioschyzon merolae* genome. A tool for facilitating comparable studies on organelle biogenesis in photosynthetic eukaryotes. *Plant Physiology* 137, 567-585.

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

Nozaki, H., M. Matsuzaki, M. Takahara, O. Misumi, H. Kuroiwa, M. Hasegawa, T. Shin-I, Y. Kohara, N. Ogasawara, and T. Kuroiwa. 2003. The phylogenetic position of red algae revealed by multiple nuclear genes from mitochondria-containing eukaryotes and an alternative hypothesis on the origin of plastids. *J. Mol. Evol.* 56, 485-497.

Palenik, B., J. Grimwood, A. Aerts, P. Rouzé, A. Salamov, N. Putnam, C. Dupont, R. Jorgensen, E. Derelle, S. Rombauts, K. Zhou, R. Otillar, S.S. Merchant, S. Podell, T. Gaasterland, C. Napoli, K. Gendler, A. Manuell, V. Tai, O. Vallon, *et al*. 2007. The tiny eukaryote *Ostreococcus* provides genomic insights into the paradox of plankton speciation. *PNAS* 104, 7705-7710.

Sato, N., S. Fujiwara, A. Kawaguchi, and M. Tsuzuki. 1997. Cloning of a gene for chloroplast Δ6 desaturase of a green alga, *Chlamydomonas reinhardtii*. *J. Biochem.* 122, 1224-1232.

Sato, N. and T. Moriyama. 2007. Genomic and biochemical analysis of lipid biosynthesis in the unicellular Rhodophyte *Cyanidioschyzon merolae*: Lack of a plastidic desaturation pathway results in the coupled pathway of galactolipid synthesis. *Eukaryotic Cell* 6, 1006-1017.

Scala, S. and C. Bowler. 2001. Molecular insights into the novel aspects of diatom biology. *Cell Mol. Life Sci.* 58, 1666-1673.

Scala, S., N. Carels, A. Falciatore, M.L. Chiusano, and C. Bowler. 2002. Genome properties of the diatom *Phaeodactylum tricornutum*. *Plant Physiology* 129, 993-1002.

Seckbach, J. 1994. The first eukaryotic cells-acid hot-spring algae. *J. Biol. Physics* 20, 335-345.

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

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

Sperling, P., P. Ternes, T.K. Zank, and E. Heinz. 2003. The evolution of desaturases. *Prostaglandins Leukot. Essent. Fatty Acids* 68, 73-95.

Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673-4680.

Tonon, T., D. Harvey, T.R. Larson, and I.A. Graham. 2002. Long chain polyunsaturated fatty acid production and partitioning to triacylglycerols in four microalgae. *Phytochemistry* 61, 15-24.

Tonon, T., O. Sayanova, L.V. Michaelson, R. Qing, D. Harvey, T.R. Larson, Y. Li, J.A. Napier, and I.A. Graham. 2005. Fatty acid desaturases from the microalga *Thalassiosira pseudonana*. *Graham FEBS J*. 272, 3401-3412.

Yoon, H.S., K.M. Müller, R.G. Sheath, F.D. Ott, and D. Bhattacharya. 2006. Defining the major lineages of red algae (Rhodophyta). *J. Phycol.* 42, 482-492.