

Isolation of a Δ^5 -Fatty Acid Desaturase Gene from *Mortierella alpina**

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Arachidonic acid (C20:4 $\Delta^{5,8,11,14}$) is a polyunsaturated fatty acid synthesized by the Δ^5 -fatty acid desaturation of di-homo- γ -linolenic acid (C20:3 $\Delta^{8,11,14}$). In mammals, it is known to be a precursor of the prostaglandins and the leukotrienes but it is also accumulated by the filamentous fungus *Mortierella alpina*. We have isolated a cDNA encoding the Δ^5 -fatty acid desaturase from *M. alpina* via a polymerase chain reaction-based strategy using primers designed to the conserved histidine box regions of microsomal desaturases, and confirmed its function by expression in the yeast *Saccharomyces cerevisiae*. Analysis of the lipids from the transformed yeast demonstrated the accumulation of arachidonic acid. The *M. alpina* Δ^5 -desaturase is the first example of a cloned Δ^5 -desaturase, and differs from other fungal desaturases previously characterized by the presence of an N-terminal domain related to cytochrome b_5 .

The filamentous fungus *M. alpina* is unusual in that it can produce a wide range of polyunsaturated fatty acids. It differs from higher plants in its fatty acid unsaturation as it is able to produce arachidonic acid (C20:4 $\Delta^{5,8,11,14}$) and eicosapentaenoic acid (C20:5 $\Delta^{5,8,11,14,17}$) (1). In filamentous fungi, polyunsaturated fatty acids are present both in the phospholipids and the triacylglycerols, indicating that they have a role in membrane structure and as storage oils (2). In animals, arachidonic acid is a precursor of the short-lived regulatory molecules, the eicosanoids, which comprise the prostaglandins, the leukotrienes, and the thromboxanes (3). All mammalian cells except erythrocytes synthesize eicosanoids, and among their many functions they are involved in inflammatory response, reproductive function, and regulation of blood pressure (4).

In fungi, unsaturated fatty acids are formed in the endoplasmic reticulum by the action of integral membrane-bound fatty acid desaturase enzymes, which sequentially insert double bonds into the acyl chain (5, 6). The Δ^5 -desaturase is responsible for the conversion of di-homo- γ -linolenic acid (C20:3 $\Delta^{8,11,14}$) to arachidonic acid. It is thought to function like the other microsomal desaturases from higher plants and yeast,

catalyzing aerobic reactions requiring cytochrome b_5 as a cofactor. Electrons are transferred from NADH-dependent cytochrome b_5 reductase, via the heme-containing cytochrome b_5 molecule to the fatty acid desaturase (7, 8).

Biochemical characterization of membrane-bound desaturases has been limited because they are difficult to purify due to their hydrophobic nature. Hence, molecular genetic approaches, particularly the use of *Arabidopsis* mutants, have provided much information on desaturation reactions (9, 10). Analysis of the predicted protein sequences for the higher plant desaturases together with those from cyanobacteria, yeast, and mammals revealed the presence of eight highly conserved histidine residues (11). Mutagenesis studies on the microsomal Δ^9 -desaturase from rat and the Δ^{12} -desaturase from *Synechocystis* (11, 12) showed that all of the conserved histidines were catalytically essential. Variation is present within the amino acid sequences between the conserved histidine residues, but sequence alignments have permitted the design of degenerate oligonucleotides capable of amplifying novel desaturase gene fragments from various sources (13–15). Although the approach has yielded gene probes for a variety of microsomal desaturase activities including Δ^6 , Δ^9 , and Δ^{12} , no information is available yet for a Δ^5 -desaturase. Here, we describe the successful application of this approach to the isolation of a Δ^5 -fatty acid desaturase from the fungus *Mortierella alpina*.

EXPERIMENTAL PROCEDURES

Cultures—*M. alpina* CBS 210.32 was grown in liquid culture in potato dextrose medium (Life Technologies, Inc.) at 28 °C with shaking at 200 rpm. After 48 h, mycelium was harvested and used in fatty acid analysis and various DNA and RNA extractions.

Mucor circinelloides was grown in liquid culture in potato dextrose medium (Life Technologies, Inc.) at 25 °C with shaking at 200 rpm. Mycelium was harvested after 48 h, and DNA was extracted.

RNA Isolation and Manipulation—Total RNA was isolated from 400 mg wet weight of mycelium using the RNeasy plant mini kit (Qiagen) and 5 μ g was reverse transcribed with the Ready-To-Go™ T-primed first strand kit (Amersham Pharmacia Biotech) according to the manufacturers' instructions. The cDNA was used as a template for PCR¹ amplification with degenerate primers.

Genomic DNA Isolation—Approximately 4 g wet weight of mycelium was ground to a fine powder under liquid nitrogen using a precooled mortar and pestle. The ground tissue was added to 10 ml of extraction buffer (10 mM Tris-HCl, pH 8, 10 mM EDTA, 0.5% SDS), thawed, and mixed gently by inversion. Ten ml of phenol:chloroform:isoamyl alcohol (16) were added and mixed gently for 15–30 min. The organic and aqueous phases were separated by centrifugation and the aqueous layer removed to a fresh centrifuge tube. This extraction was repeated until the interface between the two phases was clear, after which an extraction with chloroform:isoamyl alcohol (24:1) was carried out. DNA was

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF054824.

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¹ The abbreviations used are: PCR, polymerase chain reaction; bp, base pair(s); GC, gas chromatography; FAME, fatty acid methyl ester; MS, mass spectroscopy.

SYND6	1	-----
BORD6	1	MBAQIKKYYITSDLELKNHDKPGDLLRSTIQGRAYDVSDWVKDHPGGSFPLKSLAGQEVTDFAF
M.A5	1	MGTDQGKTFTEWELAAHNTKGDLELAIRGRVYDVTKELSRHPGGVDTLLLAGRQVTPVE
SYND6	1	-----MLTAERIKETQKRGRVFNQRVDAYEAEHGLTQRDNEM
BORD6	61	VAEHPASTWKN-LDKFFETCYLLKDYSSVEVS---KD--YRKEVFEFSKMGLYDKKGHIM
M.A5	61	EMVHAFGAADAIMKKYIVCTIVSNELPVEPEPTVVFHKTKTRVEGYFEDRDIDPKNREI
SYND6	41	YLKTLIIVLWLFSAWAFVLEAPVIFP---VRLGCMVLAIALAFSENMGHDANHNAYS
BORD6	114	FATLFCFIAFLFAMSVYVLFCEGVIV---HLFSCLMGFLWVQSGW-RGHDAQHYMVVS
M.A5	121	WGRYALIFGSLIASYYAQLVFFVWERTWLOVVFATIMGFACQVGLNPLHDASHESVWH
SYND6	98	NPHINKVGLGTYDFV--GLISFLWYRHNLYLHHTYTNIHLGHVETVHGDD--AVRMSPEOE
BORD6	169	DSRLNKFYGFFAANCLSGTISIGWKKNNHN-AHHIACNSLEYDPPQYIPFLVVSSEKFFGS
M.A5	181	NPTVWKLGLGATHDFNFN-CASYLVWVYCHMLGHPYTNLAGADPDMSTFEFDPVRRKFNCK
SYND6	154	HVGIIRFQOQFYTWGLYLFYFPFY--WFLYDNYLVLNKGMVHDHKKIFFQPLELJASLLGKIL
BORD6	228	LTSHEYEKRTTFDSLSRFVVSQYHWTFFYFIMCAARINMYVQSLIMLLKRNVSYRAHELL
M.A5	240	WVFNHINODMEVPPFLYGLLAEK--VRQDINLIFYVFKTNDAIRVNPSTSWHTVMFWGCKA
SYND6	212	LWLGIVFGLPLALG-FSTPEVVLIGASVTYMYGIVVCTIFMLAHVLESTEFLTPDGESCA
BORD6	288	GCLVESITWYPLLVSCLPNWGERIMEVITASLSVTCMQOQVQESLNHFSSSVYVGKFK----
M.A5	298	FEVWRLIVPLIQY--LPLGKVLLEFFVADNVSSYWLATTFQANHVMEVQWPLPD-ENGI
SYND6	271	LDDEWAICQIRRTANFATNNPFWNWFCCGLNHQVTHHLFPNICHVHYEYQENIKDYCOE
BORD6	343	-GNNWFEKOTEGTLDLISCP-PMDWVHGGLDFOLEBHLFPKVPKCNLRKISPYVLELCKK
M.A5	355	IQKDWAAQVETTDQMAHDSHLWTSITGSLNYQAVHHLFPNVSQHHYFDLALITKNTQSE
SYND6	331	AGVEYKVPYPTFKAAIASNYRWLEAMGKAS-----
BORD6	401	HNIIPYN-YAASFKANEMTLRTRLRNTLQARBITKPLPKNLVWEALHTHG
M.A5	415	IKVPPYLKDTFWQAFASHLEHLRVGLRPKEE-----

FIG. 1. Comparison of deduced amino acid sequences. The translation of the coding sequence of L11.1 (labeled *M.A5*) is aligned with the cyanobacterial Δ^6 -desaturase (*SYND6*) and the borage Δ^6 -desaturase (*BORD6*). Identical or conserved residues are shaded, and the conserved histidines are underlined. The sequence of L11.1 has been deposited in the GenBank data base (accession no. AF054824).

concentrated by ethanol precipitation and purified by two rounds of equilibrium centrifugation in a CsCl ethidium bromide density gradient. The band containing genomic DNA was removed from the gradient and diluted with TE buffer. DNA was recovered by ethanol precipitation and dissolved in TE buffer.

PCR-based Cloning—Highly degenerate primers were synthesized using inosine for 4-base redundancy and extended at the 5' ends to include *EcoRI* restriction sites to facilitate cloning PCR products. The forward primer was, 5'-GCGAATTC(A/G)TIGGICA(T/C)GA(T/C)TG(T/C)GGICA-3', and the reverse primer was 5'-GCGAATTCATIT(G/T)IGG(A/G)AAIA(G/A)(A/G)TG(A/G)TG-3', where I stands for inosine and the *EcoRI* sites are underlined. The primers were used for PCR amplification of cDNA reverse transcribed from total RNA. After initial denaturation at 94 °C for 2 min, amplification was performed in 32 cycles of 45 s at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, followed by a final extension at 72 °C for another 10 min. Amplification products were fractionated on 1% agarose gels from which selected DNA bands were purified. They were ligated directly into pGEM-T (Promega) and the plasmids used to transform into *Escherichia coli* DH5 α cells. Plasmid DNA was purified for sequencing using the Qiagen QIAprep mini-prep kit. Nucleotide sequences were determined using a Perkin Elmer ABI-377 DNA sequencer, and analyzed using the University of Wisconsin GCG software package (17).

Library Screening—End-adapted cDNA was synthesized from *M. alpina* poly(A)⁺ mRNA using a cDNA synthesis kit (Amersham Pharmacia Biotech) and ligated into the *EcoRI* site of λ MOSSlox (Amersham Pharmacia Biotech). The resultant DNA was packaged into phage particles to produce a library of 4 \times 10⁶ plaque-forming units with an average insert size of 1.4 kilobases. The library was screened by standard techniques (16) using cloned PCR products as probes. DNA fragments were labeled with [α -³²P]dCTP using the Ready To GoTM DNA labeling reaction mix (Amersham Pharmacia Biotech). The full-length cDNA clone L11.1 was purified by successive rounds of plating and hybridization before it was subjected to plasmid excision *in vivo* and the insert was sequenced on both strands.

Southern Blot Analysis—Aliquots (10 μ g) of genomic DNA from *M.*

alpina and *M. circinelloides* were digested with restriction enzymes and fractionated by agarose gel electrophoresis. DNA was transferred to a Zeta-Probe[®] nylon membrane (Bio-Rad) by alkaline capillary transfer for 5–8 h using 0.4 M NaOH as transfer buffer. To improve transfer of the larger DNA fragments (greater than 4 kilobases), the gel was soaked in 0.25 M HCl for 15 min to partially depurinate the DNA and then briefly rinsed in water prior to blotting. The filters were probed with the 660-bp fragment amplified between histidine boxes 1 and 3 using the gene-specific primers 5'-CATGATGCGTCTCACITTTCA-3' (forward) and 5'-(G/A)GGTGCACAGCCTGGTAGTT-3' (reverse). Overnight hybridization was performed at 55 °C in 0.25 M sodium phosphate pH 7.2, 7% SDS, after which the filters were washed in 2 \times SSC, 0.1% SDS at the hybridization temperature and x-ray film exposed to it at -80 °C using an intensifying screen (18).

Functional Analysis: Yeast Transformation—PCR with the primers MYfor, 5'-GCGGGTACCATGGGTACGGACCAAGGA-3' (annealing to the initiating methionine indicated by the boldface type), and MYrev, 5'-GCGGAGCTCCTACTCTTCTTGGGACG-3' (annealing to the complement of the stop codon, indicated in boldface type) was used to amplify the pL11 coding region and to flank it with *KpnI* and *SacI* restriction sites. The amplified PCR product was ligated into the vector pYES2 (Invitrogen) to generate the plasmid pYES2/L11 and cloned in *E. coli*. The fidelity of the cloned PCR product was checked by *in vitro* transcription and translation using the TNTTM system (Promega). Translation products labeled with [³⁵S]methionine were generated, separated by SDS-polyacrylamide gel electrophoresis, and visualized by autoradiography. The plasmid was transformed into *Saccharomyces cerevisiae* DBY746 by the lithium acetate method (19), and expression of the transgene was induced by the addition of galactose to 1% (w/v). The yeast culture medium was supplemented with 0.5 mM di-homo- γ -linolenic acid or 0.5 mM linoleic acid in the presence of 1% tertgitol as described by Napier *et al.* (20).

Fatty Acid Analysis—Total fatty acids extracted from yeast cultures were analyzed by gas chromatography (GC) of methyl esters. Lipids were transmethylated with 1 M HCl in methanol at 80 °C for 1 h, then fatty acid methyl esters (FAMES) were extracted in hexane. GC anal-

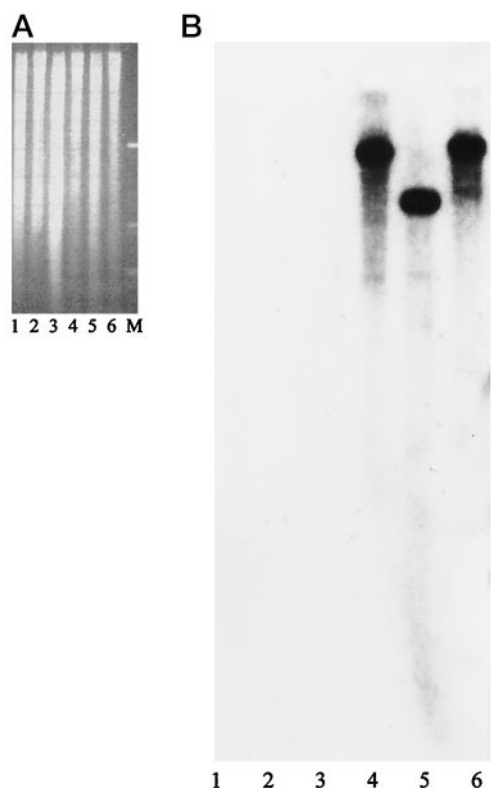


FIG. 2. Hybridization of L11.1 to genomic DNA from *M. alpina* and *M. circinelloides*. Samples of genomic DNA from *M. circinelloides* (lanes 1–3) and *M. alpina* (lanes 4–6) were digested with *Eco*RI (lanes 1 and 4), *Bam*HI (lanes 2 and 5), and *Hind*III (lanes 3 and 6). A is the 1 \times TAE, 1% agarose gel stained with ethidium bromide ($M = 100$ -bp marker, Boehringer Mannheim). B is the autoradiograph of the Southern blot of this gel probed with the fragment amplified between histidine boxes 1 and 3.

ysis of FAMES was conducted using a Hewlett Packard 5880A Series gas chromatograph equipped with a 25 m \times 0.32-mm RSL-500 BP bonded capillary column and a flame ionization detector. Fatty acids were identified by comparison with retention times of FAME standards (Sigma). Relative percentages of the fatty acids were estimated from peak areas. Arachidonic acid was identified by GC-MS using a Krats MS80RFA operating at an ionization voltage of 70 eV, with a scan range of 500–40 daltons.

RESULTS

Cloning of a *M. alpina* Membrane-bound Desaturase—The strategy employed to obtain probes for *M. alpina* desaturase genes involved reverse transcriptase PCR with the degenerate primer sequences used to amplify a plant Δ^6 -desaturase gene (15). Inosine was used at all positions of 4-base degeneracy to maximize the relative concentrations of individual primers within the degenerate mixtures. The forward primer mixture was designed to encode histidine box 1, and the reverse primer mixture encoded the complement of histidine box 3. Total RNA extracted from *M. alpina* was reverse-transcribed, and the cDNA product was used as a template in a PCR that yielded products of various sizes. Several PCR products were generated, and those of the expected length (600–700 bp) were isolated from a gel for cloning and sequencing. The DNA sequence of one of the clones (L11) predicted an open reading frame of 224 amino acids. It displayed some similarity to other membrane-bound desaturases, including the presence of the second characteristic histidine box (11). Alignment of the deduced amino acid sequence of this PCR product with the corresponding region of known desaturases indicated the greatest similarity to the Δ^6 -desaturase from a cyanobacterium (21), although the actual level of identity was low and less than 25%.

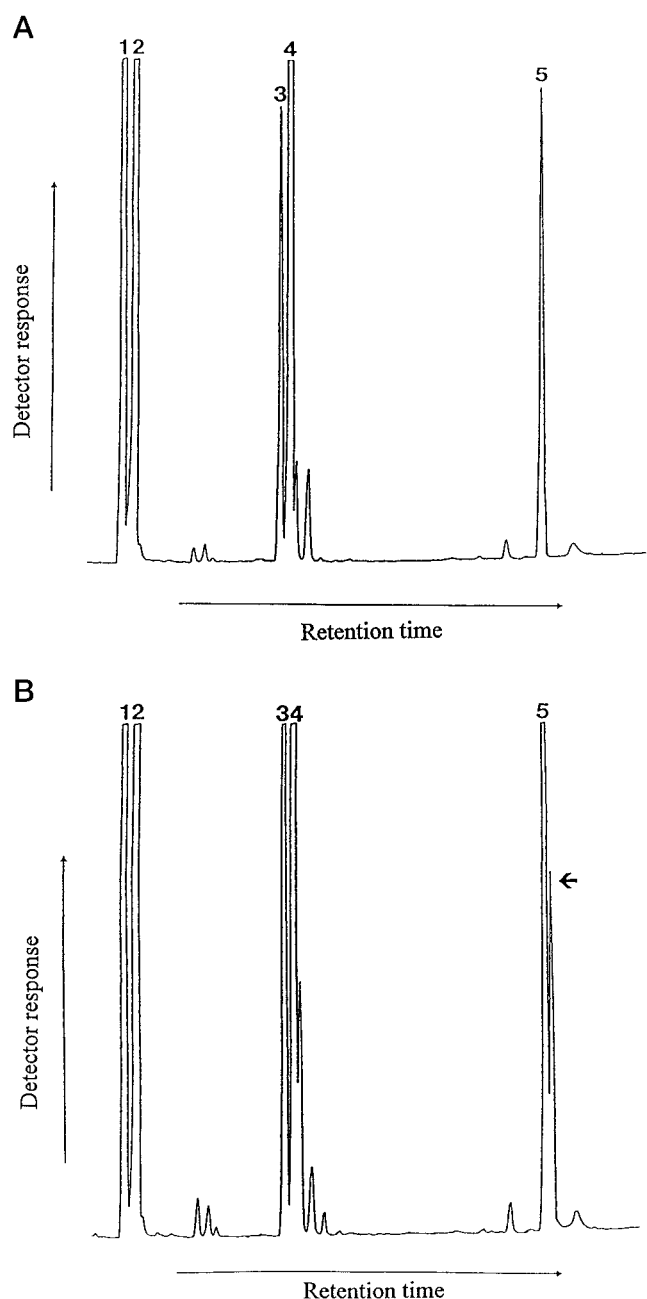


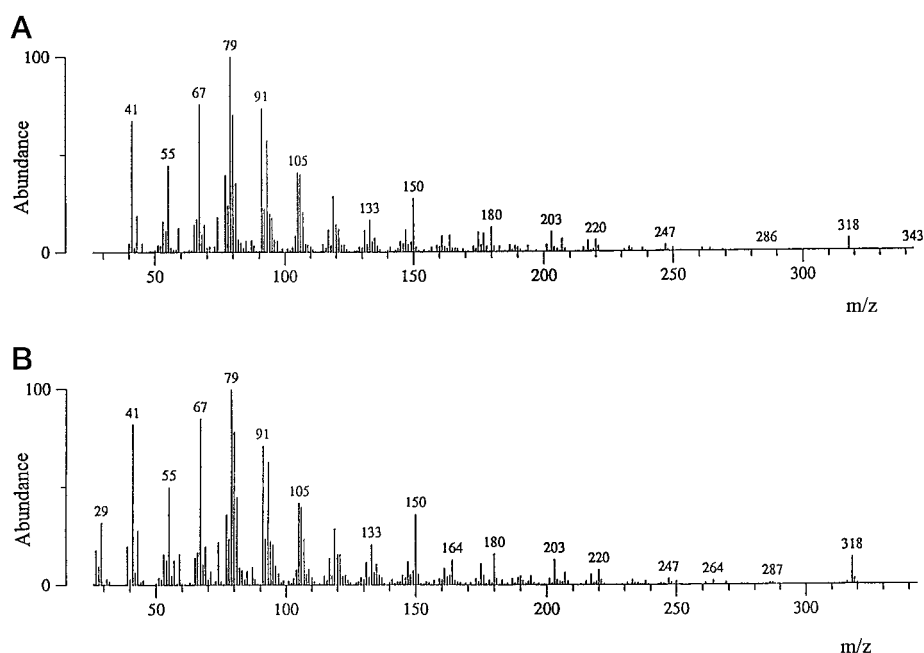
FIG. 3. Identification of arachidonic acid synthesized in transgenic yeast by GC. Fatty acid methyl esters of total lipids of *S. cerevisiae* grown under inducing conditions in the presence of di-homo- γ -linolenic acid were analyzed by GC, using flame ionization detection. A, FAMES extracted from yeast transformed with control (empty) vector pYES2; B, FAMES extracted from yeast transformed with pYES2/L11. The common peaks were identified as C16:0 (peak 1), C16:1 (peak 2), C18:0 (peak 3), C18:1 (peak 4), and C20:3 (peak 5 supplied exogenously). The additional peak (in B), which corresponds to the retention time of arachidonic acid, is indicated by the arrow.

Lipid analysis has shown that in cultures of *M. alpina* harvested after 48 h arachidonic acid comprises up to 25% of the total lipid.² It was therefore considered possible that L11 could encode part of either a Δ^6 - or a Δ^5 -desaturase from *M. alpina*.

To isolate a complete coding region corresponding to the L11 PCR product, the insert was used to probe a *M. alpina* cDNA library that had been constructed in λ MOSSlox. A sample of the primary library containing 1.48×10^5 plaque-forming units

² L. V. Michaelson, C. M. Lazarus, G. Griffiths, J. A. Napier, and A. K. Stobart, unpublished data.

FIG. 4. GC-MS analysis of the novel peak identified in yeast carrying pYES2/L11. A comparison of the mass spectra of the novel peak (A) and an authentic arachidonic acid standard (B) is shown. Visual and computer-based inspection indicate that the two spectra are identical.



was plated and screened for hybridization to L11. Fifteen individual phage plaques gave positive signals; they were cored out of the agar plates and eluted into SM buffer. The resultant phage suspensions were checked for the presence of desaturase-encoding cDNAs by PCR with the degenerate histidine box primers. Clone L11.1 was determined as having the longest insert in a PCR screen involving the histidine box 3 primer and vector arm (T7 and SP6) primers, and was purified by additional rounds of plating and hybridization screening. The plasmid pL11.1 was released from λ clone L11.1 by *in vivo* excision, and the cDNA insert was sequenced.

The 1410-bp cDNA insert of clone pL11.1 includes a 1341-bp reading frame encoding 446 amino acids and a TAG stop codon. The coding region is flanked by 3 bp from the 5'-untranslated region of the mRNA and the full 3'-untranslated region, followed by part of the poly(A) tail. Fig. 1 shows the deduced amino acid sequence of the predicted coding region. It shows 22% identity with the Δ^6 -desaturase from the cyanobacterium *Synechocystis* (21) and 20% identity with the *Borago officinalis* (borage) Δ^6 -desaturase (15). The three conserved histidine boxes that are characteristic of other membrane desaturases are present. The third histidine box contains a H \rightarrow Q substitution, which is also evident in the *B. officinalis* and the *Caenorhabditis elegans* Δ^6 -desaturase sequences (15, 20).

L11.1 Encodes a Protein with a Cytochrome b_5 -like Heme-binding Domain at the N Terminus—A heme containing electron donor is required for fatty acid desaturation and cytochrome b_5 fulfills this function for the membrane-bound desaturases (5, 8, 22). Fig. 1 shows the presence of a heme binding region characterized by the H-P-G-G motif toward the N terminus and hence the cytochrome b_5 and desaturase may exist as a fusion protein. Previously identified fungal desaturases also contain cytochrome b_5 fusions, but with the heme-binding region located toward the C terminus (5, 14).

Southern Blot Analysis—*M. circinelloides* is a filamentous zygomycete, which accumulates a triacylglycerol oil rich in γ -linolenic acid but lacking arachidonic acid. In all other respects, it is very similar to *M. alpina* (6). It thus has genes encoding Δ^9 -, Δ^{12} -, and Δ^6 -desaturase activities and is likely to lack a Δ^5 -desaturase gene. Genomic DNA was isolated from *M. alpina* and *M. circinelloides*, digested with three restriction enzymes and fractionated by agarose gel electrophoresis. A

Southern blot of the gel was probed with the 660-bp fragment amplified by PCR between histidine boxes 1 and 3 of clone L11. Fig. 2 shows single hybridizing bands in each of the digests of *M. alpina* DNA, but no hybridization was observed to *M. circinelloides* DNA. In a similar experiment, a putative Δ^9 -desaturase cDNA was found to hybridize strongly to both the *M. circinelloides* genome as well as the *M. alpina* genome.² These results indicate that the desaturase isolated from *M. alpina* is encoded by a single copy gene that is not present in *M. circinelloides*; this suggests that the gene is likely to encode a Δ^5 -desaturase rather than a Δ^6 -desaturase.

Functional Analysis of L11.1 in Yeast—The complete coding region (446 amino acids) of L11.1 was amplified by PCR and inserted into the yeast expression vector pYES2 downstream of the *GAL1* promoter. This construct was transformed into *E. coli*. The fidelity of the PCR-generated insert in plasmid pYES2/L11 was confirmed *in vitro* by coupled transcription/translation under the control of the T7 RNA polymerase promoter in the vector. The translation products were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. A product of $M_r \sim 56,000$ was produced from the plasmid pYES2/L11, whereas the empty vector control (pYES2) failed to yield any protein products (data not shown).

For functional analysis of the L11.1 coding region, the recombinant plasmid was transferred to yeast. Cells were cultured overnight in a medium containing raffinose as a carbon source, and supplemented by the addition of either linoleic acid (18:2 $\Delta^9,12$) or di-homo- γ -linolenic acid (C20:3 $\Delta^{8,11,14}$). These fatty acids are not present in *S. cerevisiae* but serve as the specific substrates for either the Δ^6 - or Δ^5 -desaturase, respectively. Expression of the L11.1 coding region from the *GAL1* promoter of the vector was induced by the addition of galactose to 1%. Growth of the cultures was continued for 16 h, when aliquots were removed for the analysis of fatty acids by GC. Fig. 3 shows the result of GC analysis of the fatty acid methyl esters of transformed yeast strains. An additional peak is apparent in the trace obtained from induced pYES2/L11 grown in the presence of di-homo- γ -linolenic acid compared with an empty vector control. This peak was also absent from uninduced cultures grown on di-homo- γ -linolenic acid and it is also important to note that pYES2/L11 grown in the presence of linoleic acid failed to accumulate any novel peaks indicating that this fatty

acid is not a substrate for the enzyme encoded by the *M. alpina* cDNA. The retention time of the additional peak is identical to that of the authentic methyl-arachidonic acid standard. The fatty acid produced from di-homo- γ -linolenic acid was further characterized by GC-MS and identified as arachidonic acid (Fig. 4). The results show, therefore, that yeast cells transformed with the plasmid pYES2/L11 had acquired functional Δ^5 -desaturase activity and were now capable of synthesizing arachidonic acid from the substrate di-homo- γ -linolenic acid. The Δ^5 -desaturase in the transformed yeast appeared to be a very efficient catalyst, with 21.3% of the substrate converted to arachidonic acid under the conditions of the experiment.

Identification of a Similar Sequence in *C. elegans*—Data base searching with the *M. alpina* cDNA identified a high scoring match to one of the genes on *C. elegans* cosmid T13F2 (GenBank accession number Z81122). The *C. elegans* ORF of T13F2.1 predicts a protein of 454 amino acids. It contains the characteristics of other membrane-bound desaturases including the three histidine box domains. It also contains an N-terminal cytochrome b_5 region, and the third histidine box contains the H \rightarrow Q variant. As the nematode performs Δ^5 desaturation (23), it is possible that this homologue encodes a Δ^5 -desaturase gene.

DISCUSSION

A cDNA isolated from *M. alpina* has been characterized and found to encode a protein with three histidine box motifs indicative of a microsomal fatty acid desaturase (11). The deduced protein sequence also contained the diagnostic features of a heme-binding cytochrome b_5 domain located toward the N terminus. Evidence that the L11.1 clone encodes a Δ^5 -desaturase was obtained by Southern blot analysis, which indicated the absence of homologous sequences in the genome of *M. circinelloides*, a related fungus that produces γ -linolenic acid but not arachidonic acid. Functional analysis of the L11.1 clone in transformed yeast confirmed that it encoded a Δ^5 -desaturase gene, since cells growing in a medium supplemented with di-homo- γ -linolenic acid produced arachidonic acid in significant quantities. The fungal Δ^5 -desaturase appears to differ from the other fungal and plant desaturases that have been characterized (5, 14). The most closely related sequence identified was that of the cyanobacterial Δ^6 -desaturase involved in GLA formation (21), with which the fungal Δ^5 -desaturase showed only some 22% amino acid identity. Data base searches have also enabled us to identify a putative animal Δ^5 -desaturase in the nematode *C. elegans*.

It is interesting that the Δ^5 -desaturase may exist as a cytochrome b_5 fusion protein. Such fusions have been identified in a hypothetical desaturase-like sunflower protein (24) and the Δ^6 -desaturase in *B. officinalis*, both of which contain N-terminal cytochrome b_5 domains (15). Δ^9 -Desaturases characterized from other fungi have also been found to contain cytochrome b_5 domains, but in these examples the diagnostic heme binding site was located toward the C terminus (5, 14). The Δ^5 -desaturase from *M. alpina*, therefore, appears to be the first fungal fatty acid desaturase described with an N-terminal cytochrome b_5 domain and in this respect is similar to the "front end" Δ^6 -desaturase of *B. officinalis* (15, 25) and the Δ^6 -desaturase of *C. elegans* (20). In contrast to other microsomal desaturases, the microsomal desaturases with N-terminal cytochrome b_5 domains use a polyunsaturated fatty acid as a substrate and insert a double bond between the methyl end of the fatty acid and existing double bonds, but it is unclear how significant a role the fused cytochrome b_5 region plays in this reaction. The *OLE1* gene isolated from *S. cerevisiae*, which encodes a Δ^9 -desaturase, contains a C-terminal cytochrome b_5 domain. *OLE1* rescued double mutants deficient in both *OLE1* and the

separate microsomal cytochrome b_5 . However, when the cytochrome b_5 region was deleted from this gene, the yeast cells remained fatty acid auxotrophs, even in the presence of the endogenous yeast cytochrome b_5 (5). This suggests that the cytochrome b_5 domain plays an essential role in the desaturation reaction. Whether the cytochrome b_5 -desaturase fusion proteins are more efficient awaits further assessment.

Recently, another N-terminal cytochrome b_5 fusion protein, Fah1p, has been identified in yeast; it is required for the α -hydroxylation of sphingolipid-associated very-long-chain fatty acids (26). Interestingly, it contains two of the histidine box motifs characteristic of membrane-bound desaturases. The *Arabidopsis* homologue of Fah1p has no cytochrome b_5 domain but is able to functionally complement a *fah1* deletion mutant. This indicates that, in the case of Fah1p, the N-terminal cytochrome b_5 may not be essential for function (26).

Desaturases isolated from fungal sources have much potential for further exploitation, particularly in the genetic manipulation of oil crops to produce novel and diverse polyunsaturated fatty acid products for the pharmaceutical and nutraceutical industries (27). The Δ^5 -desaturase is an essential enzyme in the production of eicosanoids, and the identification of a *M. alpina* cDNA encoding this activity is the first example of its type.

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