cDNA cloning and characterization of human Δ^5-desaturase involved in the biosynthesis of arachidonic acid

Amanda E. LEONARD*, Bruce KELDER†, Emil G. BOBIK*, Lu-Te CHUANG*, Jennifer M. PARKER-BARNES*, Jennifer M. THURMOND*, Paul E. KROEGER‡, John J. KOPCHICK†, Yung-Sheng HUANG* and Pradip MUKERJI*[1]

*Department of Strategic Discovery Research, Ross Products Division, Abbott Laboratories, 3300 Stelzer Road, Columbus, OH 43219, U.S.A., †Department of Biomedical Sciences, Ohio University, Edison Biotechnology Institute, Athens, OH 45701, U.S.A., and ‡Department of Genomics and Molecular Biology, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, U.S.A.

Two human expressed sequence tag (EST) cDNA sequences with identity with Δ^5- and Δ^6-desaturases from a filamentous fungus, *Mortierella alpina*, were identified from the LifeSeq® database of Incyte Pharmaceuticals, Inc. (Palo Alto, CA, U.S.A.). An oligonucleotide complementary to the 3′ EST cDNA sequences was used to screen human liver cDNA using rapid amplification of cDNA ends (RACE)–PCR. The amplified DNA fragment had 98 % identity with a putative open reading frame (ORF) predicted from a human genomic sequence, and encoded 444 amino acids. Expression of this ORF in mouse fibroblast cells demonstrated that the encoded protein was a Δ^5-desaturase, as determined by the conversion of dihomo-γ-linolenic acid ($C_{20:3,n-6}$) into arachidonic acid ($C_{20:4,n-6}$). The human Δ^5-desaturase contained a predicted N-terminal cytochrome b_5-like domain, as well as three histidine-rich domains. A tissue expression profile revealed that this gene is highly expressed in fetal liver, fetal brain, adult brain and adrenal gland. A search of the existing databases led to localization of this ORF within a 14 kb interval flanked by the flap endonuclease-1 (FEN1) and vitelliform macular dystrophy (Best's disease; VMD2) loci of chromosome 11q12.

Key words: chromosome 11, dihomo-γ-linolenic acid (DGLA), long-chain polyunsaturated fatty acids.

INTRODUCTION

Long-chain polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA; $C_{20:4,n-6}$) and docosahexaenoic acid ($C_{22:6,n-3}$), are important components of infant as well as adult nutrition, serving as structural elements of cell membranes. Long-chain PUFAs containing 20 carbon atoms also serve as precursors to eicosanoids, including prostaglandins and prostacyclins [1], and play key roles in various biological functions, such as the inflammatory response [2], fetal growth and development [3], retina function [4] and brain development [5]. In the human, the two main families of long-chain PUFAs, $n-6$ and $n-3$, are derived either from the diet or from the metabolism of dietary linoleic acid ($C_{18:2,n-6}$) and α-linolenic acid ($C_{18:3,n-3}$). Both $C_{18:2,n-6}$ and $C_{18:3,n-3}$ are desaturated by Δ^6-desaturase. The product of Δ^6-desaturation, γ-linolenic acid (GLA; $C_{18:3,n-6}$) or stearidonic acid ($C_{18:4,n-3}$), is then converted by an elongase into dihomo-γ-linolenic acid (DGLA; $C_{20:3,n-6}$) or $C_{20:4,n-3}$ respectively. These two fatty acids are then converted by a Δ^5-desaturase into AA and eicosapentaenoic acid (EPA; $C_{20:5,n-3}$) respectively.

The Δ^6-desaturase has been isolated and characterized from different species, including plants [6], moss [7], nematode [8], and recently human [9]. Tissue distribution analysis of human Δ^6-desaturase revealed that there are high mRNA levels in brain, liver, heart and lung [9]. The human Δ^6-desaturase contains membrane-spanning domains, a cytochrome b_5-like domain and three conserved histidine-rich domains [9]. The Δ^5-desaturase has also been isolated and characterized from several sources, including bacteria [10], fungus [11] and nematode [12]. In the present paper we describe the isolation of a desaturase-like sequence from human liver and demonstrate the ability of the encoded enzyme to produce Δ^5-desaturated fatty acids in mammalian cells. We also compare the tissue distribution and chromosomal location of the human Δ^5- and Δ^6-desaturases.

MATERIALS AND METHODS

Cloning of the human Δ^5-desaturase cDNA

The *Mortierella alpina* Δ^5-desaturase and Δ^6-desaturase cDNA sequences were used to search the LifeSeq® database of Incyte Pharmaceuticals, Inc. The search revealed two human expressed sequence tag (EST) sequences (LifeSeq® Clone ID numbers 3350263 and 3448789) that had identity with the middle and C-terminal sections of the translated fungal Δ^5- and Δ^6-desaturase sequences. The clusters (groups of clones related to one another by sequence identity) containing these sequences were identified (Cluster ID numbers 2511785 and 3506132). The edited consensus sequences of the two clusters were imported into the Sequencher software program (Gene Codes Corp.) and assembled to create a sequence of 1843 bases, designated 2535.

Primer RO430 (5′-GTG GCT GTT GTT ATT GGT GAA GAT AGG CAT C-3′), specific for 3′ region of sequence 2535, was utilized to screen the human liver Marathon-Ready® cDNA, using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA, U.S.A.). The termini of the 1.4 kb PCR-amplified product were filled-in with T4 DNA polymerase (Boehringer

Abbreviations used: AA, arachidonic acid; BAC, bacterial artificial chromosome; bGH, bovine growth hormone; DGLA, dihomo-γ-linolenic acid; EPA, eicosapentaenoic acid; EST, expressed sequence tag; GLA, γ-linolenic acid; ORF, open reading frame; PUFA, polyunsaturated fatty acid; QPCR, quantitative PCR; RACE, rapid amplification of cDNA ends.

[1] To whom correspondence should be addressed (e-mail pradip.mukerji@rossnutrition.com).

The nucleotide sequence data reported will appear in the GenBank®, GSDB, EMBL and DDBJ Nucleotide Sequence Databases under the accession number AF226273.

Mannheim, Indianapolis, IN, U.S.A.), the DNA fragment was cloned into the pCR®-blunt vector (Invitrogen, Carlsbad, CA, U.S.A.), and the resulting plasmid was designated A-1.

To generate a full-length cDNA, two primers that were modified with 5′ phosphate, RO526 (5′-*P*CAT GGC CCC CGA CCC GGT GG-3′) and RO527 (5′-*P*GCG GCC ACC GGG TCG GGG GC-3′), were annealed to form an adaptor. This adaptor, containing the extending termini corresponding to *Nco*I and *Bsa*I, was ligated with the gel-purified A-1 (*Bsa*I/*Hin*dIII) cDNA fragment at room temperature for 15 min. The pYX242 (*Nco*I/*Hin*dIII) vector (Novagen, Madison, WI, U.S.A.) was added to this ligation mixture and allowed to incubate at room temperature for an additional 45 min. The resulting plasmid was designated pRAE-28-5.

A 1335 bp *Nco*I/*Hin*dIII DNA fragment encoding the putative human Δ^5-desaturase cDNA was isolated from plasmid pRAE-28-5, and the termini of the cDNA were filled-in with Klenow polymerase (Stratagene, La Jolla, CA, U.S.A.). The filled-in cDNA was ligated into plasmid pMTK-bGH-C (*Bgl*II/*Pvu*II), and the resulting plasmid was designated pMET-h Δ^5-desaturase-bGHpA. This plasmid utilizes the mouse metallothionein I transcription regulatory element to direct human Δ^5-desaturase transcription and the bovine growth hormone (bGH) polyadenylation signal for proper processing of the 3′ terminus of the mRNA.

Expression of human Δ^5-desaturase in mouse L cells

Mouse L cells were transfected with the pMET-h Δ^5-desaturase-bGHpA plasmid by the calcium phosphate precipitation procedure to generate stable cell lines [13]. Individual L cell clones were analysed for the presence of integrated desaturase sequences by DNA slot-blot hybridization analysis, using a ^{32}P-radiolabelled DNA probe containing sequences from bGH exon V and 3′ untranslated region. The resulting clones were screened for desaturase activity in serum-containing medium and in serum-free medium with or without added substrates. GLA and DGLA were used as substrates for the determination of expressed cDNA activity. L cells without the human Δ^5-desaturase cDNA were used as a negative control.

Fatty acid analysis

The fatty acid distribution (as percentage of total fatty acids) in mammalian cells containing the human Δ^5-desaturase cDNA, in the presence of various substrates, was analysed using the method described previously [11].

Tissue distribution analysis

In order to quantify the amounts of Δ^5- and Δ^6-desaturase mRNAs in a variety of human tissues, we employed Taqman© Real Time QPCR (quantitative PCR) methodology using the ABI Prism 7700 (Perkin-Elmer Applied Biosystems, Foster City, CA, U.S.A.). Samples for QPCR analysis were prepared by synthesizing first-strand cDNA from 5 μg of total RNA (from each of 22 human tissue samples) using the Superscript II kit as described by the manufacturer (Life Technologies, Gaithersburg, MD, U.S.A.). Based on the sequence alignment between the Δ^5- and Δ^6-desaturases, gene-specific Taqman© primers and probes were designed using the Primer Express program (Perkin-Elmer ABI) and synthesized with standard phosphoramidite chemistry. All Taqman© probes were 5′-labelled with the reporter fluorescein (denoted below as [FAM]) and 3′-labelled with the quencher tetramethylrhodamine (denoted below as [TAMRA]).

Δ^5-Desaturase sequences used for primer design were as follows: forward primer 374F, 5′-TGA AGG CCA ACC ATG TCT TCT-3′; reverse primer 441R, 5′-CCA GGC TGC ACC ATC CAG-3′; Taqman© probe 396T, 5′-[FAM]CCT GCT GTA CCT GCT GCA CAT CTT GCT[TAMRA]. Δ^6-desaturase sequences used for primer design were as follows. Set 1: forward primer 462F, 5′-TGG CAA TGG CTG GAT TCC TA-3′; reverse primer 526R, 5′-CAG CTT GGG CCT GAG AGG T-3′; Taqman probe 483T, 5′-[FAM]CCT CAT CAC GGC CTT TGT CCT TGC[TAMRA]-3′. Set 2: forward primer 1015F, 5′-CAG ATG AAT CAC ATC GTC ATG GA-3′; reverse primer 1089R, 5′-GGT GGC TGT CAG CTG GCT A-3′; Taqman probe 1040T, 5′-[FAM]TTG ACC AGG AGG CCT ACC GTG ACT G[TAMRA]-3′.

Each QPCR reaction was prepared in triplicate as follows. Final concentrations of reagents were: 1× Taqman buffer (Perkin-Elmer Applied Biosystems), 5 mM $MgCl_2$, 200 nM forward and reverse primers, 100 nM Taqman probe, 200 μM dNTPs and 0.625 unit of Amplitaq Gold in a final reaction volume of 25 μl. A portion of 10 ng of target cDNA was added to each reaction, which were then placed in an Optical PCR plate (Perkin-Elmer Applied Biosystems) for thermal cycling. QPCR reaction conditions were one cycle for 10 min at 95 °C, followed by 40 cycles of 94 °C for 15 s and 60 °C for 1 min. Data were collected continuously during the PCR reaction and analysed with the ABI-7700 SDS software package. The point at which amplification resulted in significant fluorescence above background was determined and noted as the threshold cycle (Ct). These Ct data were then used to normalize and calculate the fold differences between Δ^5- and Δ^6-desaturase mRNAs in the human tissues. We used a variation of the Comparative or $\Delta\Delta$Ct method (Perkin-Elmer Applied Biosystems Prism 7700 Users Bulletin Number 2). Initially, the absolute content of cDNA added to each reaction was normalized with a 28 S rRNA QPCR control and compared with the 28 S rRNA content of a gold standard of human embryonic kidney cDNA used in the laboratory Subsequently, normalized fold differences were determined by comparison of all samples amplified with a particular desaturase primer set to the cerebellum sample in that sample group (cerebellum fold difference = 1.0).

Location of human Δ^5- and Δ^6-desaturases on chromosome 11

The human chromosomal sequence, AC004770, was identified as a human Δ^5-desaturase homologue by searching the GenEMBL database (GCG, Madison, WI, U.S.A.). Between 50 and 100 amino acid segments of both human Δ^5- and Δ^6-desaturase [9] translated sequences were used as a query to search this chromosomal sequence, which was translated in six different reading frames. All of the exons that comprise the Δ^5- and Δ^6-desaturases were identified on this bacterial artificial chromosome (BAC) sequence. The orientations of the human Δ^5- and Δ^6-desaturase genes were also established.

RESULTS

Cloning of human Δ^5-desaturase

The LifeSeq® database was searched using the amino acid sequences of the *M. alpina* Δ^5-desaturase and Δ^6-desaturase cDNA sequences. Sequence 2535, which had 27 % identity with the fungal Δ^5-desaturase and 26.7 % identity with the fungal Δ^6-desaturase, was generated (see the Materials and methods section). The GenEMBL database (GCG) search with the cDNA sequence from A-1 as the query revealed that the A-1 sequence had 94.6 % identity in 203 bp overlap with the human genomic

Table 1 Fatty acid profiles of mouse L cells containing the human Δ^5-desaturase and control cells

Values are averages of two determinations.

Fatty acid	Composition (%, by wt.)	
	Control L cells	Containing human Δ^5-desaturase
$C_{14:0}$	0.94	0.65
$C_{16:0}$	14.88	11.08
$C_{18:0}$	14.73	14.73
$C_{20:0}$	0.38	0.35
$C_{22:0}$	0.37	0.61
$C_{24:0}$	1.18	0.46
$C_{18:2,n-6}$	4.31	8.06
$C_{20:2,n-6}$	0.30	1.25
$C_{20:3,n-6}$ (DGLA)	0.35	0.65
$C_{20:4,n-6}$ (AA)	1.34	4.74
$C_{22:4,n-6}$	0.21	1.01
$C_{18:3,n-3}$	0.15	0.23
$C_{20:5,n-3}$ (EPA)	0.19	0.32
$C_{22:5,n-3}$	0.44	1.42
$C_{22:6,n-3}$	0.26	0.93

sequence AC004770. This DNA sequence was from *Homo sapiens* chromosome 11, BAC CIT-HSP-311e8 (BC269730). Clone A-1 did not contain a translational start codon; the open reading frame (ORF) in clone A-1 appeared to be missing 18 bp at the 5′ end when compared with the putative ORF in AC004770. The complete open reading frame of the putative cDNA in pRAE-28-5, including the 18 bp, was 1335 bp.

Expression of Δ^5-desaturase in L cells

Two transfected mouse L cell clones containing integrated human Δ^5-desaturase sequences were obtained. To meet the need for essential fatty acids, both control (L) cells and human Δ^5-desaturase transfected cells were grown in serum-containing medium. The cells were analysed for Δ^5-desaturase activity by determining the levels of various fatty acids, including PUFAs, within the cells. The results are summarized in Table 1.

In this study, AA and EPA were the predicted products of human Δ^5-desaturase activity. The mouse L cell clones containing stably integrated human Δ^5-desaturase sequences contained significantly elevated levels of AA and EPA, with average increases of 254 % and 68 % respectively compared with those in control L cells. Furthermore, the levels of other downstream $n-6$ and $n-3$ PUFAs were found to be significantly elevated in these cells. The average increases were 381 % in the level of $C_{22:4,n-6}$, 223 % in the level of $C_{22:5,n-3}$ and 258 % in the level of $C_{22:6,n-3}$. The levels of $C_{18:2,n-6}$, $C_{20:2,n-6}$ and $C_{18:3,n-3}$ were also higher than in the control cells.

To demonstrate further the human Δ^5-desaturase activity, the control and stably transfected mouse L cell clones were incubated in serum-free culture medium with or without 100 μM GLA (Table 2). In serum-free medium without GLA, the levels of DGLA were similar in control and human Δ^5-desaturase-containing cells, while the levels of GLA were undetectable. However, the levels of AA and $C_{22:4,n-6}$ were increased by 152 % and 137 % respectively in the Δ^5-desaturase-containing cells. The levels of $C_{18:2,n-6}$ and $C_{20:2,n-6}$ were also higher than in the control cells, while $C_{18:3,n-3}$ was not detected. Addition of GLA to the serum-free culture medium resulted in an accumulation of GLA in both control and human Δ^5-desaturase-containing cells. The levels of DGLA were also elevated in both cell types, suggesting that the exogenous GLA was rapidly elongated by an active endogenous elongase. However, in comparison with the control cells, the level of DGLA was 53 % lower, while the levels of AA and $C_{22:4,n-6}$ were 79 % and 270 % higher respectively, in the clones containing human Δ^5-desaturase. The levels of $C_{18:2,n-6}$, $C_{20:2,n-6}$ and $C_{18:3,n-3}$ were also higher than in the control cells.

To confirm the human Δ^5-desaturase activity, the control and the stably transfected mouse L cell clones were also incubated in serum-free culture medium with 100 μM DGLA (Table 2). Addition of DGLA to the serum-free culture medium resulted in the accumulation of DGLA in both control and human Δ^5-desaturase-containing cells. However, in comparison with the control cells, the level of DGLA was 79 % lower, while the levels of AA and $C_{22:4,n-6}$ were 24 % and 135 % higher respectively, in the human Δ^5-desaturase-containing clones. The levels of $C_{18:2,n-6}$ and $C_{20:2,n-6}$ were lower than those in the control cells, while that of $C_{18:3,n-3}$ was slightly higher. To demonstrate this desaturase activity further, GLC analysis of one of the mouse L cell clones against a control L cell is shown in Figure 1. All evidence indicated that this cDNA expressed an active human Δ^5-desaturase.

Characterization of the pRAE-28-5 cDNA

The three 'histidine boxes' known to be conserved among membrane-bound desaturases were found at amino acid positions 179–183, 216–220 and 382–386 of the putative polypeptide

Table 2 Fatty acid profiles of mouse L cells containing human Δ^5-desaturase and control cells, grown under three different conditions

Cells were grown in the absence of serum in all cases. Values are averages of two determinations. ND, not detected.

Fatty acid	Composition (%, by wt.)					
	No additions		+ 100 μM GLA ($C_{18:3,n-6}$)		+ 100 μM DGLA ($C_{20:3,n-6}$)	
	Control cells	+ Human Δ^5-desaturase	Control cells	+ Human Δ^5-desaturase	Control cells	+ Human Δ^5-desaturase
$C_{18:2,n-6}$	3.50	7.55	3.00	4.61	6.19	5.66
$C_{20:2,n-6}$	0.28	0.74	0.54	0.92	0.55	0.15
$C_{18:3,n-6}$ (GLA)	ND	ND	1.41	3.04	0.14	0.18
$C_{20:3,n-6}$ (DGLA)	0.36	0.36	7.34	3.48	18.19	3.82
$C_{20:4,n-6}$ (AA)	1.32	3.33	12.54	22.48	14.13	17.51
$C_{22:4,n-6}$	0.08	0.19	2.21	8.18	2.59	6.09

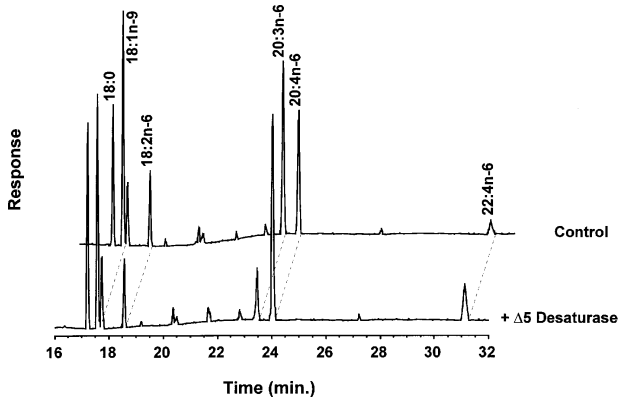

Figure 1 GLC analysis of fatty acid methyl esters from the lipid fractions of mouse L cells containing human Δ^5-desaturase and control L cells

$+\Delta^5$ Desaturase, results from the Δ^5-desaturase-containing cells.

encoded by the ORF (Figure 2). Similar to other membrane-bound desaturases, the final HXXHH histidine-box motif was found to be QXXHH [11]. The cytochrome b_5-like domain present in the translated pRAE-28-5 cDNA sequence had 32.2% identity with the human cytochrome b_5 translated sequence [14].

The database was searched with the translated human desaturase cDNA sequence as a query, and the sequences with the highest percentage identity were human Δ^6-desaturase (62% identity) [9], mouse Δ^6-desaturase (60% identity) [9], rat Δ^6-desaturase (60% identity) [15] and moss Δ^6-desaturase (28% identity) [7]. The FastA alignment results with *M. alpina*

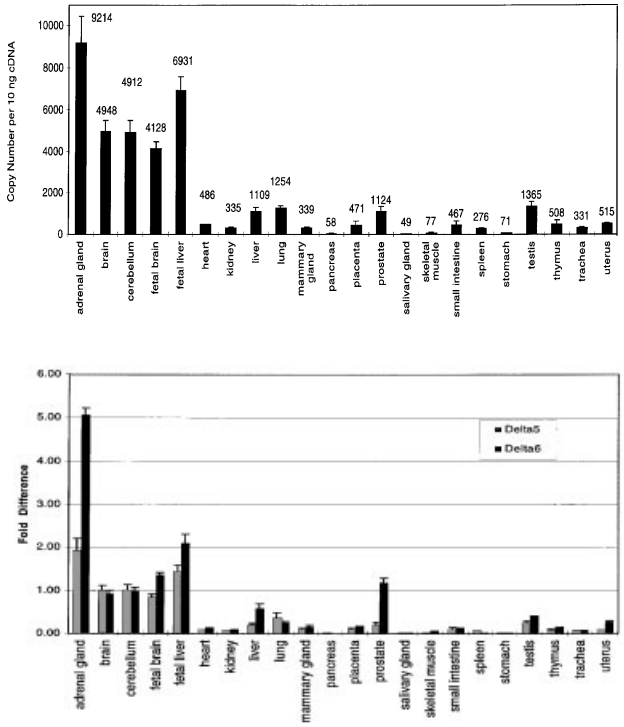

Figure 3 Tissue distribution of human Δ^5-desaturase

The abundance of expressed Δ^5-desaturase in various human tissues is shown. Upper panel: the absolute content of cDNA added to each reaction was normalized with a 28 S rRNA QPCR control. Lower panel: normalized fold differences were determined by comparison of all samples amplified with a particular desaturase primer set to the cerebellum sample (= 1.0) in that sample group. The average data for the two primer sets were used for Δ^6-desaturase.

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hdelta5   1  ----MAPDPVAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNI  45
hdelta6   1  MGKGGNQGEGAAEREVSVPT---FSWEEIQKHNLRTDRWLVIDRKVYNI  46

hdelta5  46  SEFTRRHPGGSRVISHYAGQDATDPFVAFHINKGLVKKYMNSLLIGELS  94
hdelta6  47  TKWSIQHPGGQRVIGHYAGEDATDAFRAFHPDLEFVGKFLKPLLIGELA  95

hdelta5  95  PEQPSFEPTKNKELTDEFRELRATVERMGLMKANHVFFLLYLLHIILLD  143
hdelta6  96  PEEPSQDHGKNSKITEDFRALRKTAEDMNLFKTNHVFFLLLLAHIIALE  144

hdelta5 144  GAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQHDFGHLSVFSTSKW  192
hdelta6 145  SIAWFTVFYFGNGWIPTLITAFVLATSQAQAGWLQHDYGHLSVYRKPKW  193

hdelta5 193  NHLLHHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFFAL  241
hdelta6 194  NHLVHKFVIGHLKGASANWWNHRHFQHHAKPNIFHKDPDVNMLHVFVL  241

hdelta5 242  GKILSVELGKQKKKYMPYNHQHKYFFLIGPPALLPLYFQWYIFYFVIQR  290
hdelta6 242  GEWQPIEYGKKKLKYLPYNHQHEYFFLIGPPLLIPMYFQYQIIMTMIVH  290

hdelta5 291  KKWVDLAWMITFYVRFFLTYVPLLG-LKAFLGLFFIVRFLESNWFVWVT  338
hdelta6 291  KNWVDLAWAVSYYIRFFITYIPFYGILGALLFLNFI-RFLESHWFVWVT  338

hdelta5 339  QMNHIPMHIDHDRNMDWVSTQLLATCNVHKSAFNDWFSGHLNFQIEHHL  387
hdelta6 339  QMNHIVMEIDQEAYRDWFSSQLTATCNVEQSFFNDWFSGHLNFQIEHHL  387

hdelta5 388  FPTMPRHNYHKVAPLVQSLCAKRGIEYQSKPLLSAFADIIHSLKESGQL  436
hdelta6 388  FPTMPRHNLHKIAPLVKSLCAKHGIEYQEKPLLRALLDIIRSLKKSGKL  436

hdelta5 437  WLDAYLHQ                                          444
hdelta6 437  WLDAYLHK                                          444
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Figure 2 Comparison of the deduced amino acid sequences of human Δ^5- and Δ^6-desaturases

Identical residues are shaded. The cytochrome b_5-like region is overlined. The three histidine-box regions are in bold. Abbreviations: hdelta5, human Δ^5-desaturase; hdelta6, human Δ^6-desaturase.

desaturases were 30.5% identity with the Δ^6-desaturase [16], 27.5% identity with the Δ^5-desaturase [11] and 19% identity with the Δ^{12}-desaturase [16].

Δ^5-Desaturase mRNA distribution in human tissues

QPCR analysis of human Δ^5-desaturase expression in 22 human tissues revealed that the highest levels of mRNA were found in the adrenal gland, fetal liver, brain, cerebellum and fetal brain (Figure 3, upper panel). Interestingly, the level in fetal liver was 6-fold greater than that found in adult liver. The same pattern of expression was seen for human Δ^6-desaturase. Figure 3 (lower panel) compares the fold differences in mRNA levels relative to the cerebellum control, comparing human Δ^5- and Δ^6-desaturases. Again, the level of human Δ^6-desaturase in fetal liver was several-fold higher than adult liver.

DISCUSSION

The cDNA sequence of human Δ^5-desaturase involved in long-chain PUFA biosynthesis was isolated based on identity among human cDNA sequences and *M. alpina* Δ^5- and Δ^6-desaturase cDNA sequences. Expression of this human cDNA in mouse fibroblast cells, grown in serum-containing medium, resulted in increased levels of many PUFAs, including $C_{22:4,n-6}$, $C_{22:5,n-3}$ and $C_{22:6,n-3}$. These increases could have resulted from higher levels of AA and EPA (produced by the recombinant Δ^5-desaturase), which were further converted by other endogenous desaturases and elongases. However, the increases in the C_{22} fatty acids could also be due to the increased accumulation of $C_{18:2,n-6}$, $C_{20:2,n-6}$ and $C_{18:3,n-3}$, which could then be converted by endogenous desaturases and elongases in addition to the recombinant human Δ^5-desaturase. Increased levels of many PUFAs were also seen when the stably transfected cells were grown in serum-free medium or serum-free medium containing GLA. Again, the increase in $C_{22:4,n-6}$ could have resulted from higher levels of $C_{18:2,n-6}$, $C_{20:2,n-6}$ and $C_{18:3,n-3}$ being converted by endogenous desaturases and elongases. However, when DGLA was used as a substrate in serum-free medium, the levels of $C_{18:2,n-6}$, $C_{20:2,n-6}$ and $C_{18:3,n-3}$ were not significantly increased in comparison with the control cells. In fact, the levels of $C_{18:2,n-6}$ and $C_{20:2,n-6}$ were lower in the L cells containing human Δ^5-desaturase cDNA than in the control cells, while conversion of DGLA into AA was much higher than in the control cells. This confirmed that the increased level of AA in transfected cells compared with the control cells was due to the activity of the recombinant human Δ^5-desaturase.

Expression of the human cDNA in mouse fibroblast cells demonstrated its ability to introduce a Δ^5 double bond into both DGLA and $C_{20:4,n-3}$. This suggests that transgenic animals may prove to be a good system for studying the physiology associated with the expression of human Δ^5-desaturase, as well as other enzymes associated with fatty acid biosynthesis pathways.

The predicted amino acid sequence encoded by human Δ^5-desaturase cDNA had the highest percentage identity with mammalian Δ^6-desaturases; more specifically, it had 62% identity with human Δ^6-desaturase [9] (Figure 2). Since these two enzymes desaturate two different carbon atoms, it would be interesting to know where the enzyme specificity is located within the protein sequences and which region of the protein is responsible for distinguishing this difference. Like the human Δ^6-desaturase sequence [9], the human Δ^5-desaturase sequence contains a cytochrome b_5-like domain, as well as three conserved histidine-rich domains. QPCR analysis of 22 human tissues revealed that Δ^5-desaturase not only is expressed in various tissues, but also is expressed at similar levels to the Δ^6-desaturase.

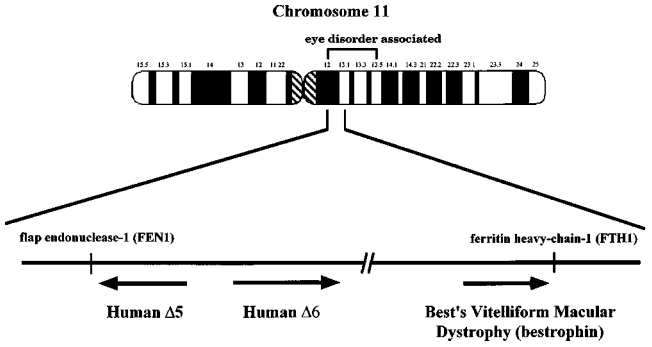

Figure 4 Localization of the human Δ^5- and Δ^6-desaturases on chromosome 11

The region associated with eye disorders is indicated with a bracket.

These levels correlate with those of the Δ^6-desaturase mRNA determined by Northern-blot analysis [9]. Similarity was also predicted from a dendrogram (results not shown); the human Δ^5-desaturase is more closely related to human Δ^6-desaturase than to any other desaturase. In comparisons with non-human desaturases, the human Δ^5-desaturase is more closely related to *Caenorhabditis elegans* Δ^5-desaturase than to the fungal Δ^5-desaturase.

These human desaturases not only are similar, but also are located close to each other on chromosome 11q12, in the region between the flap endonuclease-1 (FEN1) and vitelliform macular dystrophy (Best's disease; VMD2) loci [17]. It is not known whether these genes evolved through duplication. The Δ^5-desaturase is more centromeric, with its 3′ sequence closer to the FEN1 locus (Figure 4). The two genes are in opposite 5′ to 3′ orientations, spanning approx. 63 kb, within the critical chromosomal region responsible for Best's disease, a well-known eye disorder [18]. There are also other eye disorders associated with chromosome 11q, including autosomal dominant familial exudative vitreoretinopathy [19], autosomal dominant neovascular inflammatory vitreoretinopathy [20], Bardet–Biedl syndrome [21] and osteoporosis--pseudoglioma syndrome [22]. More recently, a variant of congenital fibrosis of the extraocular muscles has been linked to chromosome 11q13.1 [23]. The mapping of the human Δ^5-desaturase to this region may suggest experiments to explore the relationship between the changes in this gene and the development of various eye disorders.

We have also identified another putative Δ^5-desaturase cDNA sequence during RACE (rapid amplification of cDNA ends)–PCR. This cDNA has been cloned, and the resulting plasmid was designated pRAE-41. The expressed cDNA in pRAE-41 converted similar levels of DGLA into AA in baker's yeast as the expressed cDNA in pRAE-28-5 (results not shown). The Δ^5-desaturase cDNA in plasmid pRAE-41, compared with the Δ^5-desaturase cDNA sequence in plasmid pRAE-28-5, encodes different amino acids at positions 361 (glutamine) and 410 (histidine) (see Figure 2). These two cDNAs could be natural variants of the Δ^5-desaturase. However, the base differences could also be due to mutations which occurred during cDNA synthesis or during RACE–PCR.

In summary, we have identified two human Δ^5-desaturase cDNAs that are very similar to the human Δ^6-desaturase. The deduced amino acid sequences of the human Δ^5-desaturases differed by only two amino acids, at positions 361 and 410. Both cDNAs expressed active Δ^5-desaturase in recombinant cells.

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