# Cloning of a human cDNA encoding a novel enzyme involved in the elongation of long-chain polyunsaturated fatty acids

Amanda E. LEONARD*, Emil G. BOBIK*, Joseph DORADO*, Paul E. KROEGER†, Lu-Te CHUANG*, Jennifer M. THURMOND*, Jennifer M. PARKER-BARNES*, Tapas DAS*, 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., and †Department of Genomics and Molecular Biology, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, U.S.A.

The *Saccharomyces cerevisiae* protein ELO2p is involved in the elongation of saturated and monounsaturated fatty acids. Among several sequences with limited identity with the *S. cerevisiae ELO2* gene, a consensus cDNA sequence was identified from the LifeSeq® database of Incyte Pharmaceuticals, Inc. Human liver cDNA was amplified by PCR using oligonucleotides complementary to the 5′ and 3′ ends of the putative human cDNA sequence. The resulting full-length sequence, termed HELO1, consisted of 897 bp, which encoded 299 amino acids. However, in contrast with the *ELO2* gene, expression of this open reading frame in *S. cerevisiae* demonstrated that the encoded protein was involved in the elongation of long-chain polyunsaturated fatty acids, as determined by the conversion of $\gamma$-linolenic acid ($C_{18:3,n-6}$) into dihomo-$\gamma$-linolenic acid ($C_{20:3,n-6}$), arachidonic acid ($C_{20:4,n-6}$) into adrenic acid ($C_{22:4,n-6}$), stearidonic acid ($C_{18:4,n-3}$) into eicosatetraenoic acid ($C_{20:4,n-3}$), eicosapentaenoic acid ($C_{20:5,n-3}$) into $\omega$3-docosapentaenoic acid ($C_{22:5,n-3}$) and $\alpha$-linolenic acid ($C_{18:3,n-3}$) into $\omega$3-eicosatrienoic acid ($C_{20:3,n-3}$). The predicted amino acid sequence of the open reading frame had only 29 % identity with the yeast ELO2 sequence, contained a single histidine-rich domain and had six transmembrane-spanning regions, as suggested by hydropathy analysis. The tissue expression profile revealed that the *HELO1* gene is highly expressed in the adrenal gland and testis. Furthermore, the *HELO1* gene is located on chromosome 6, best known for encoding the major histocompatibility complex, which is essential to the human immune response.

Key words: arachidonic acid, chromosome 6, eicosapentaenoic acid, $\alpha$-linolenic acid, $\gamma$-linolenic acid.

## INTRODUCTION

Long-chain polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA; $C_{20:4,n-6}$) and docosahexaenoic acid (DHA; $C_{22:6,n-3}$), are important for neonatal growth and brain development [1,2]. AA, an $n-6$ fatty acid, is a constituent of membrane phospholipids involved in signal transduction, and can be utilized for eicosanoid synthesis [3]. DHA, an $n-3$ fatty acid, is the main component of the PUFAs in retina and brain [4]. It has been shown that DHA intake is associated with increased visual acuity in infants, whereas DHA deficiency is associated with cognitive decline and the onset of Alzheimer's disease in adults [5].

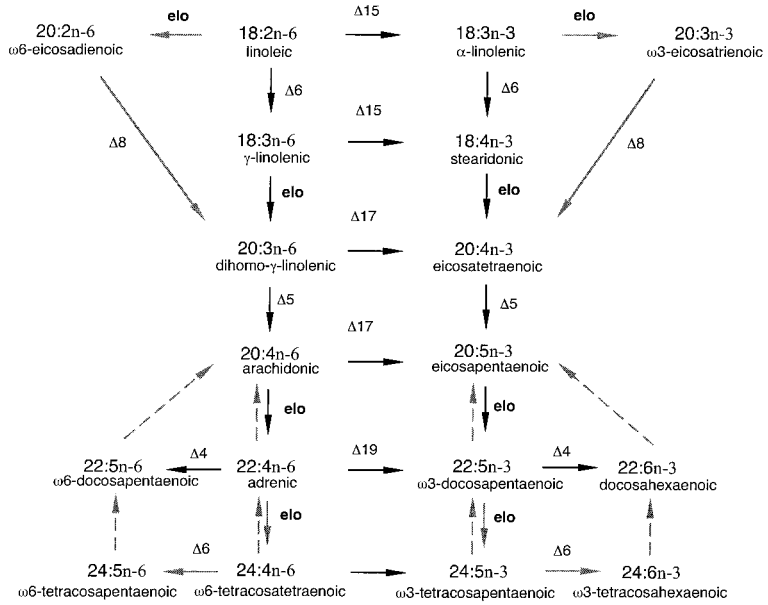
The $n-6$ and $n-3$ long-chain PUFAs are either obtained directly from the diet or derived from metabolism of dietary linoleic acid (LA; $C_{18:3,n-6}$) and $\alpha$-linolenic acid (ALA; $C_{18:3,n-3}$). In animal tissues, $\Delta^6$-desaturase converts LA into $\gamma$-linolenic acid (GLA; $C_{18:3,n-6}$), to which an elongase system adds two carbon atoms to synthesize dihomo-$\gamma$-linolenic acid (DGLA; $C_{20:3,n-6}$) (Scheme 1). In the $n-3$ pathway, $\Delta^6$-desaturase converts ALA into stearidonic acid (STA; $C_{18:4,n-3}$), to which an elongase system adds two carbons to synthesize eicosatetraenoic acid (ETA; $C_{20:4,n-3}$). A $\Delta^5$-desaturase converts DGLA and ETA into AA and eicosapentaenoic acid (EPA; $C_{20:5,n-3}$) respectively. Further elongation of EPA by two carbons results in the synthesis of $\omega$3-docosapentaenoic acid (DPA; $C_{22:5,n-3}$). It has generally been agreed that DHA is synthesized through conversion of DPA by a $\Delta^4$-desaturase. However, recent studies indicate that DHA can also be produced through an alternative pathway involving two elongation steps, from EPA to DPA and from DPA to $\omega$3-tetracosapentaenoic acid (TPA; $C_{24:5,n-3}$). This is followed by a $\Delta^6$-desaturation [TPA to $\omega$3-tetracosahexaenoic acid (THA; $C_{24:6,n-3}$)], and retroconversion from THA into DHA by $\beta$-oxidation in the peroxisomes [6]. Similarly, in the $n-6$ pathway, AA can be elongated to adrenic acid (ADA; $C_{22:4,n-6}$), and ADA to $\omega$6-tetracosatetraenoic acid (TTA; $C_{24:4,n-6}$). The TTA is $\Delta^6$-desaturated to $\omega$6-tetracosapentaenoic acid ($\omega$6-TPA; $C_{24:5,n-6}$), and $\omega$6-TPA is then retroconverted into $\omega$6-docosapentaenoic acid ($\omega$6-DPA; $C_{22:5,n-6}$) [6]. Overall, formation of $n-6$ and $n-3$ long-chain PUFAs from their precursors LA and ALA requires at least three elongation steps and three or more different desaturases capable of utilizing long-chain PUFAs as substrates.

The fatty acid chain elongation system (FACES) in animals is a four-enzyme system consisting of condensing enzyme, $\beta$-oxoacyl-CoA reductase, $\beta$-hydroxyacyl-CoA dehydrase and

Abbreviations used: GLA, $\gamma$-linolenic acid; DGLA, dihomo-$\gamma$-linolenic acid ; AA, arachidonic acid; DHA, docosahexanoic acid; LA, linoleic acid; ADA, adrenic acid; STA, stearidonic acid; ETA, eicosatetraenoic acid; EPA, eicosapentaenoic acid; DPA, $\omega$3-docosapentaenoic acid; ALA, $\alpha$-linolenic acid; ETrA, $\omega$3-eicosatrienoic acid; EDA, $\omega$6-eicosadienoic acid; EA, eicosenoic acid; TPA, $\omega$3-tetracosapentaenoic acid; THA, $\omega$3-tetracosahexaenoic acid; TTA, $\omega$6-tetracosatetraenoic acid; $\omega$6-TPA, $\omega$6-tetracosapentaenoic acid; $\omega$6-DPA, $\omega$6-docosapentaenoic acid; PUFA, polyunsaturated fatty acid; FACES, fatty acid chain elongation system; QPCR, quantitative PCR; EST, expressed sequence tag.

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

The nucleotide sequence data reported will appear in the GenBank®, EMBL, DDBJ and GSDB Nucleotide Sequence Databases under the accession number AF231981.




**Scheme 1 Metabolic pathways of long-chain PUFAs**

The common pathway for the synthesis of $n-6$ and $n-3$ $C_{22}$ fatty acids are shown with bold arrows, alternative pathways are shown with grey arrows, and retroconversion is shown with broken arrows.

trans-2-enoyl-CoA reductase [7]. Although FACES has been found in many tissues, the many forms of FACES, i.e. multiple condensing enzymes connected to a single pathway or to multiple pathways, are still unknown [8]. An animal fatty acid synthase has been characterized for shorter-chain-length saturated fatty acids [9]. More recently, very-long-chain (22+ carbon atoms) acyl-coA synthetase from human liver was described to play a role in elongation of saturated very-long-chain fatty acids or complex-lipid synthesis [10]. Although chain-length-specific PUFA-elongating enzymes were suggested to exist in rat microsomes [11], no enzyme or enzyme systems responsible for the elongation of long-chain PUFAs have been identified from animals.

The yeast *ELO1* gene, the product of which is involved in the elongation of $C_{14:0}$ to $C_{16:0}$, was first identified in 1996 [12]. The *ELO2* and *ELO3* genes were identified later, based on identity with the *ELO1* gene [13]. ELO2 protein is involved in the elongation of saturated and monounsaturated fatty acids up to 24 carbons in length, while ELO3 protein has a preference for a broader group of saturated and monounsaturated fatty acids, and is essential for the conversion of $C_{24:0}$ into $C_{26:0}$ [13]. All three proteins contain at least five membrane-spanning regions and a single histidine-box motif [13]. However, each of these three genes is thought to encode a single enzyme component of one or more systems that elongate saturated and monounsaturated, fatty acids, but not PUFAs.

Despite the identification of genes involved in the elongation of saturated and monounsaturated fatty acids from many organisms, no gene specific for elongation of long-chain PUFAs has been identified. In this paper we describe the isolation of a cDNA sequence, HELO1, from a human liver cDNA pool and demonstrate the ability of the encoded enzyme, HELO1p, to produce elongated long-chain PUFAs when expressed in bakers' yeast. We also report the distribution of the HELO1 transcript in various human tissues.

## MATERIALS AND METHODS

### Identification and cloning of the HELO1 cDNA

The *Saccharomyces cerevisiae ELO2* DNA sequence was used as a query to search the LifeSeq® database of Incyte Pharmaceuticals, Inc. (Palo Alto, CA, U.S.A.). The search revealed a cluster (groups of clones related to one another by sequence identity) of human EST (expressed sequence tag) sequences (LifeSeq® Cluster ID number CC067284R1) which exhibited sequence identity with *ELO2*. The edited consensus sequence of the cluster was then imported into the Sequencher software program (Gene Codes Corporation) and assembled to create a sequence of 897 bases, designated HELO1.

Primers RO719 (5′-GGT TCT CCC ATG GAA CAT TTT GAT GCA TC-3′) and RO720 (5′-GGT TTC AAA GCT TTG ACT TCA ATC CTT CCG-3′) were designed based on the putative HELO1 sequence, and used to amplify human liver Marathon-Ready® cDNA. The termini of the approx. 960 bp by PCR-amplified product were filled-in with T4 DNA polymerase (Boehringer Mannheim, Indianapolis, IN, U.S.A.). The DNA fragment was then cloned into the pCR®-blunt vector (Invitrogen, Carlsbad, CA, U.S.A.), and the resulting plasmid was designated pRAE-52. The HELO1 sequence was isolated from this plasmid by *Nco*I/*Hin*dIII digestion and ligated into the pYX242 (*Nco*I/*Hind*III) vector (Novagen, Madison, WI, U.S.A.) to form another plasmid, designated pRAE-58.

### Expression of the human cDNA in bakers' yeast

The plasmid DNA, pRAE-58, was transformed into *S. cerevisiae* 334 and the resulting clones were screened for elongase activity [14]. *S. cerevisiae* 334 containing the pYX242 vector alone was used as the negative control. The cultures were grown for 44 h at 30 °C in selective media containing individual substrates. GLA

was used as the substrate for the initial screening. Later, different substrates such as AA, STA, EPA and ALA were also used to determine the activity of the expressed cDNA.

### Fatty acid analysis

The yeast cell pellets were harvested and total lipids were extracted. The fatty acid distribution (as percentage of total fatty acids) in recombinant yeast cells containing either pRAE-58 or pYX242, in the presence of various substrates, was analysed using the method described previously [14]. Percentage conversion was calculated as [product/(substrate + product)] × 100.

### Distribution of HELO1 mRNA

In order to quantify the amount of HELO1 mRNA in different human tissues, Taqman® Real Time QPCR (Quantitative PCR) methodology was used as previously described [15]. HELO1 sequences used for primer design were as follows: forward primer 818F, 5′-CCA TGG CTG CTG TGA ATG G-3′; reverse primer 886R, 5′-GCT TCC TTG GCT TCA CAT TGT T-3′; Taqman® probe 838T, 5′-[FAM]CAC ACC AAC AGC TTT TCA CCC CTG GA[TAMRA] (where FAM denotes fluorescein and TAMRA denotes tetramethylrhodamine).

## RESULTS

### Identification of a putative human protein sequence involved in the elongation of fatty acids

The *S. cerevisiae ELO2* translated sequence was used as a query to search the LifeSeq® database for a putative human protein sequence involved in long-chain PUFA elongation. The first of the two resulting full-length sequences consisted of 897 bp, which encoded a protein of 299 amino acids. Hydropathy analysis using the TMpredict algorithm suggested that this putative human protein, HELO1p, contained six transmembrane regions (Figure 1). The initial membrane-scanning region is located 32 residues from the start codon. A histidine box, often present in membrane-bound enzymes [16], was found between predicted transmembrane regions III and IV, at amino acid positions 143–147 of the putative polypeptide encoded by the open reading frame (Figure 1).

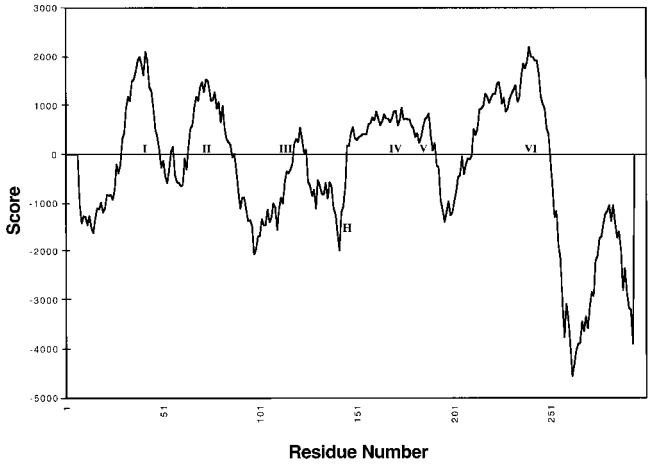



**Figure 1 TMpredict plot showing hydrophobicity and presumptive membrane-spanning regions of the HELO1 protein coding sequence**

Roman numerals indicate presumptive membrane-spanning regions. The region containing the histidine motif is indicated by H.

The translated HELO1 sequence exhibited 29.1 % identity with the *S. cerevisiae* ELO2 sequence (Figure 2) and 27.7 % identity with *S. cerevisiae* ELO3 [13]. No other known enzymes involved in the elongation of fatty acids, such as acyl-CoA synthetase [11] or fatty acid synthase [17], were found to have any significant identity with the HELO1 sequence.

The second resulting full-length sequence consisted of 837 bp, which encoded 279 amino acids. This putative human protein, HELO2p, had 27.3 % and 27.7 % identity with *S. cerevisiae* ELO2 and ELO3 respectively (results not shown).

### Expression of the human cDNAs in yeast

To demonstrate the two-carbon elongation of a long-chain PUFA, the recombinant yeast strain 334(pRAE-58) was grown in minimal medium supplemented with 25 $\mu$M of a long-chain PUFA substrate, GLA. The results in Figure 3 show that the fatty acid profile of the yeast strain 334(pRAE-58) was significantly different from that of the control strain, 334(pYX242). In particular, a fatty acid with a retention time identical with that of DGLA in GC accumulated to a level of 7.29 % of total fatty acids in strain 334(pRAE-58). The ion fragmentation pattern of this fatty acid was found to be identical with that of an authentic DGLA standard by GC–MS (results not shown). This peak represented 78.3 % conversion of the substrate fatty acid GLA into DGLA. This finding clearly demonstrated that GLA was elongated by two carbon atoms, and that HELO1p was indeed involved in the elongation of this PUFA substrate.

The overexpression of the *S. cerevisiae* ELO2 cDNA in bakers' yeast in the presence of GLA resulted in 3.8 % conversion into DGLA, which was not significant compared with the 3.7 % conversion in the control strain (results not shown). The expression of HELO2p in bakers' yeast in the presence of GLA resulted in 2.4 % conversion into DGLA, which was not significant compared with the 1 % conversion in the control strain (results not shown). Based on these results, it appears that neither *S. cerevisiae* ELO2 nor human HELO2p are involved in the elongation of GLA.

The yeast cells expressing the recombinant HELO1 sequence, when compared with the control cells, also contained significantly elevated levels of $C_{18:1,n-7}$ and $C_{20:1,n-7}$, and to a lesser extent of eicosenoic acid (EA; $C_{20:1,n-9}$) (Figure 3). This finding suggested that the recombinant HELO1p might also be involved in the elongation of monounsaturated fatty acids of 16 or 18 carbons. To confirm this hypothesis, 25 $\mu$M exogenous oleic acid was added as a substrate to the recombinant yeast strain 334(pRAE-58). After incubation, the accumulation of EA to represent 2.25 % of total fatty acids demonstrated that the expressed HELO1 enzyme can also increase the elongation of monounsaturated fatty acids (Table 1). However, the percentage conversion of oleic acid into EA by recombinant HELO1p was only 8.9 %; this was significantly lower than the endogenous conversion of $C_{16:1,n-7}$ (into $C_{18:1,n-7}$) or $C_{18:1,n-7}$ (into $C_{20:1,n-7}$), which were 20.4 % and 58.1 % respectively.

To test for the elongation of other PUFA substrates in the presence of this enzyme, the recombinant yeast strain 334 (pRAE-58) was grown in minimal medium containing another $n-6$ fatty acid (AA) or an $n-3$ fatty acid (STA, EPA or ALA) at a concentration of 25 $\mu$M. The lipid profiles of these yeast cultures, when examined by GC and GC–MS, indicated that there was accumulation of ADA, ETA, DPA and $\omega$3-eicosatrienoic acid

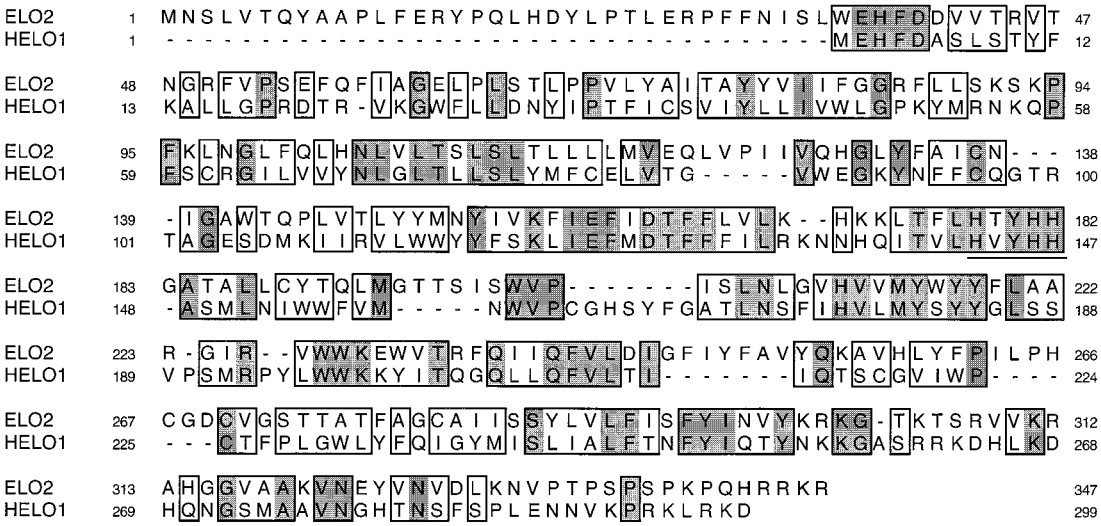



**Figure 2 Comparison of the deduced amino acid sequences of HELO1 and yeast ELO2**

Identical residues are shaded in dark grey, and similar residues are boxed. The histidine-box region is underlined.

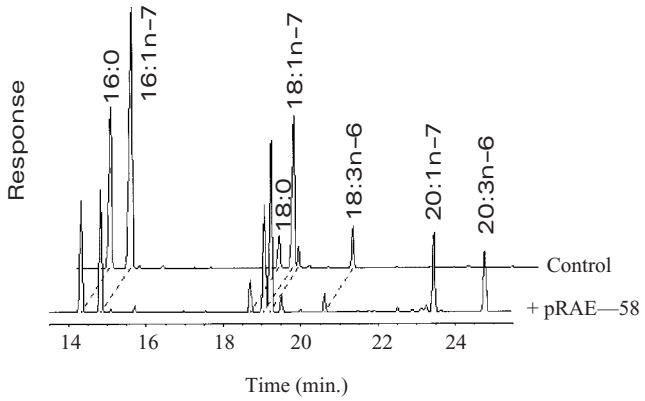



**Figure 3 GC analysis of fatty acid methyl esters from the lipid fraction of yeast cells containing pRAE-58 or control plasmid**

The results from the extracts of HELO1-expressing cells are denoted with + pRAE-58.

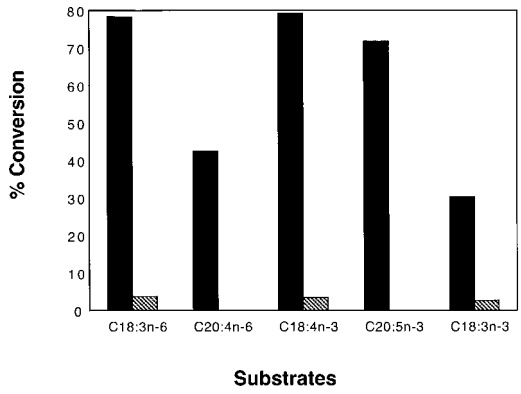



**Figure 4 Fatty acid analysis of yeast cells containing pRAE-58 and of control cells, when grown in the presence of various substrates**

The cultures were grown in the presence of the indicated substrates. Percentage conversion was calculated as [product/(substrate + product)] × 100. Results show percentage conversion of various substrates by yeast cells containing pRAE-58 (solid bars) and by yeast cells containing the control plasmid (hatched bars).

**Table 1 Fatty acid profiles of yeast cells containing pRAE-58 and control cells, grown in the presence of 25 $\mu$M oleic acid**

| Fatty acid | Content (% by wt.) | |
|---|---|---|
| | pRAE-58 | Control |
| $C_{16:1,n-7}$ | 12.87 | 35.62 |
| $C_{18:1,n-7}$ | 16.42 | 2.50 |
| $C_{20:1,n-7}$ | 9.35 | 0.10 |
| $C_{18:1,n-9}$ | 23.06 | 29.42 |
| $C_{20:1,n-9}$ | 2.25 | 0.27 |

(ETrA; $C_{20:3,n-3}$) respectively (Figure 4). The levels of these fatty acids represented 6.26 % (ADA), 10.06 % (ETA), 6.66 % (DPA), and 6.15 % (ETrA) of the total fatty acids in the strain containing the pRAE-58 sequence. This represented 42.7 %, 79.2 %, 71.7 % and 30.4 % conversion respectively of the substrate fatty acids into the product elongated by two carbon atoms. All results confirmed that the expression of HELO1 from human liver in yeast resulted in the elongation of various long-chain PUFAs in $n-6$ and $n-3$ fatty acid pathways.

## HELO1 mRNA distribution in human tissues

QPCR analysis of HELO1 expression in 22 human tissues revealed that the highest levels of HELO1 mRNA were found in

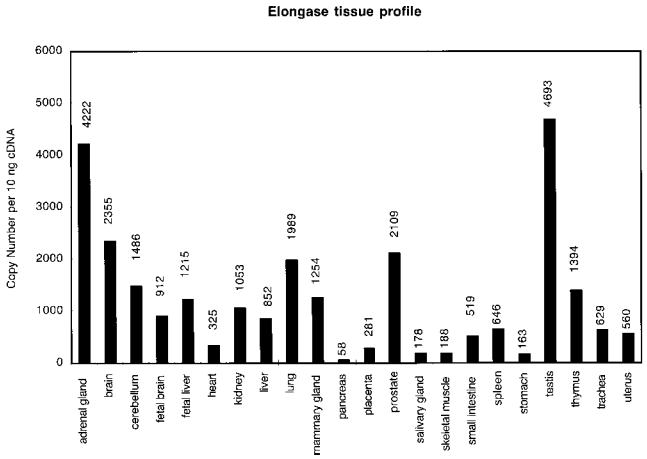



**Figure 5 Tissue distribution of HELO1**

The abundance of expressed HELO1 in various human tissues is shown. The absolute content of cDNA added to each reaction was normalized with a 28 S rRNA QPCR control.

the testis and adrenal gland (Figure 5). Other significant amounts of this mRNA were found in brain, lung and prostate tissue, each of which had approximately the same level. In these tissues, copy numbers of this mRNA per 10 ng of cDNA were approx. 50 % of that found in testis and adrenal gland. The level of HELO1 mRNA was lowest in pancreas tissue (approx. 36-fold lower than that found in brain, lung and prostate).

## DISCUSSION

In the present study, the initial search for an enzyme involved in elongation of long-chain PUFAs began by examining known saturated and monounsaturated FACES. The animal fatty acid synthase had been characterized for shorter-chain-length saturated fatty acids [9], and a very-long-chain acyl-coA synthetase from human liver was described to play a role in elongation of saturated very-long-chain fatty acids [10]. Also, chain-length-specific PUFA-elongating enzymes were suggested to exist in rat microsomes [11]. However, no enzyme or enzyme systems responsible for the elongation of long-chain PUFAs had been identified from animals. The yeast *ELO2* and *ELO3* genes were identified based on greater than 70 % similarity to the *ELO1* gene [13]. These three gene products are all involved in the elongation of fatty acids, but they have different substrate preferences. ELO1 protein is involved in the elongation of $C_{14:0}$ to $C_{16:0}$, while ELO2 and ELO3 are involved in the elongation of very-long-chain saturated and monounsaturated fatty acids [13]. Yeast mutants defective in $C_{12:0}$ elongation (mutated *ELO1*) had unimpaired elongation of $C_{16:0}$, indicating that yeast may contain different elongation systems and that other components of elongation are unaffected by this gene mutation [18].

Our approach was to identify a gene whose product is involved in the elongation of long-chain PUFA substrates, unlike yeast *ELO2*, which could be expressed in yeast. The HELO1 cDNA sequence, encoding a protein involved in the biosynthesis of long-chain PUFAs, was identified based on the limited identity between the *S. cerevisiae* ELO2 and human EST sequences. When the HELO1 cDNA was expressed in yeast cells, its protein product was involved in the conversion of a wide range of exogenously added long-chain PUFA substrates into their respective elongated products: GLA into DGLA, AA into ADA, STA into ETA, EPA into DPA and ALA into ETrA (Figure 4). This human enzyme was also involved in the elongation of endogenous monounsaturated fatty acids to their respective elongated products, and thus exhibited a broad range of substrate specificity. However, when *S. cerevisiae* ELO2 and human HELO2 were expressed in yeast cells, neither was involved in significant conversion of GLA into DGLA.

The elongation of ALA to ETrA seems to follow an alternative pathway for the synthesis of ETA, bypassing the requirement for $\Delta^6$-desaturase. In the $n-3$ pathway, ETrA is converted into ETA by a $\Delta^8$-desaturase [19] (Scheme 1). Although LA was not tested as a substrate in our study, it is likely that the recombinant HELO1p can add two carbons to LA to produce $\omega$6-eicosadienoic acid (EDA; $C_{20:2,n-6}$) in bakers' yeast. Albert et al. [20] have shown that EDA was used as a precursor of AA in the $n-6$ pathway in human testes. It is interesting that the $\Delta^8$-desaturation pathway was present in human testis [20], glioma [21] and breast cancer cells [22], but not in corresponding non-cancerous breast cells [22] or in the brain [23]. The elongation of ALA to ETrA is the first step by which $C_{20}$ fatty acids can be metabolized in tissues with little or no $\Delta^6$-desaturase activity [22]. Hence HELO1p may be essential in the biosynthesis of DGLA or $C_{20:4,n-3}$ in either pathway involving $\Delta^8$-desaturation.

As shown in Scheme 1, an alternative fatty acid biosynthesis pathway for ADA or DPA involves elongase, $\Delta^6$-desaturase and retroconversion activities [6]. ADA and DPA were added exogenously to test whether they could be elongated in yeast cells expressing HELO1p. Although ADA and DPA were effectively taken into the cells and reached as much as 49.89 % and 56.17 % respectively of total lipid, no $C_{24}$ product was detected in any of the experiments (results not shown). Therefore the recombinant form of this human enzyme is not involved in the elongation of $C_{22}$ PUFAs.

When the predicted amino acid sequence encoded by HELO1 was compared with those of other enzymes known to be involved in the elongation of fatty acids, the human enzyme showed 29.1 % identity with *S. cerevisiae* ELO2 (Figure 2) and 27.7 % identity with *S. cerevisiae* ELO3 [13]. Although all three enzymes appear to be essential for the elongation of fatty acids by two carbon atoms, the identity of the protein sequences between the two species is limited. It is interesting to note that a translated mouse EST sequence (GenBank® accession no. AI787925) had 92.5 % identity in a 106-amino-acid overlap with the HELO1 translated sequence. This high percentage identity between these two species suggests that a homologue of human HELO1p is also present in the mouse. To date, the domain of the enzyme which is responsible for substrate specificity, i.e. the ability to distinguish among saturated, monounsaturated and polyunsaturated fatty acids, has not yet been determined.

The tissue distribution of the HELO1 transcript in 22 different human tissues revealed that the highest levels of mRNA were found in the testis and adrenal gland (Figure 5). These results are consistent with the fact that both tissues contain very high levels of ADA, which is synthesized by elongation of AA [24,25].

*HELO1* is located on chromosome 6p12, centromeric to the major histocompatibility complex, which spans approx. 4 Mb on the short arm of the chromosome (6p21.3) [26]. Chromosome 6 is also believed to encode genes involved in genetic diseases, including arthritis [27], diabetes [28], haemochromatosis [29], schizophrenia [30], juvenile myoclonus epilepsy [31], multiple sclerosis [32], anterior eye chamber anomalies [33], Parkinson's disease [34], psoriasis [35], inflammatory bowel disease [36], and others. It is interesting that the *HELO1* gene is mapped to this region, since $n-3$ and $n-6$ fatty acids, as well as the ratio between the two groups of fatty acids, have been associated with

eye disorders [37], diabetes, inflammatory disorders, and many other diseases [38]. Further experiments are needed to study the regulation of *HELO1* gene expression in various disease conditions.

In summary, we have identified two human genes that have limited identity with the yeast *ELO2* and *ELO3* genes. Expression of HELO1 cDNA in bakers' yeast produced an active enzyme that is involved in the elongation of PUFAs and monounsaturated fatty acids, while expression of HELO2 cDNA in bakers' yeast produced an enzyme that was not involved in the elongation of PUFA.

## REFERENCES


1 Carlson, S. E., Werkman, S. H., Peeples, J. M., Cooke, R. J. and Tolley, E. A. (1993) Arachidonic acid status correlates with first year growth in preterm infants. Proc. Natl. Acad. Sci. U.S.A. **90**, 1073–1077

2 Uauy, R., Birch, E., Birch, D. and Peirano, P. (1992) Visual and brain function measurements in studies of n-3 fatty acid requirements of infants. J. Pediatr. **120**, S168–S180

3 Spector, A. A. (1999) Essentiality of fatty acids. Lipids **34**, S1–S3

4 Crawford, M. A., Costeloe, K., Ghebremeskel, K., Phylactos, A., Skirvin, L. and Stacey, F. (1997) Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? Am. J. Clin. Nutr. **66**, 1032s–1041s

5 Horrocks, L. A. and Yeo, Y. K. (1999) Health benefits of docosahexaenoic acid. Pharmacol. Res. **40**, 211–225

6 Sprecher, H. (1999) An update on the pathways of polyunsaturated fatty acid metabolism. Curr. Opin. Clin. Nutr. Metab. Care **2**, 135–138

7 Suneja, S. K., Nagi, M. N., Cook, L. and Cinti, D. L. (1991) Decreased long-chain fatty acyl CoA elongation activity in quaking and jimpy mouse brain: deficiency in one enzyme or multiple enzyme activities? J. Neurochem. **57**, 140–146

8 Cinti, D. L., Cook, L., Nagi, M. N. and Suneja, S. K. (1992) The fatty acid chain elongation system of mammalian endoplasmic reticulum. Prog. Lipid Res. **31**, 1–51

9 Smith, S. (1994) The animal fatty acid synthase: one gene, one polypeptide, seven enzymes. FASEB J. **8**, 1248–1259

10 Steinberg, S. J., Wang, S. J., McGuinness, M. C. and Watkins, P. A. (1999) Human liver-specific very-long-chain acyl-coenzyme A synthetase: cDNA cloning and characterization of a second enzymatically active protein. Mol. Genet. Metab. **68**, 32–42

11 Luthria, D. L. and Sprecher, H. (1997) Studies to determine if rat liver contains multiple chain elongating enzymes. Biochim. Biophys. Acta **1346**, 221–230

12 Toke, D. A. and Martin, C. E. (1996) Isolation and characterization of a gene affecting fatty acid elongation in Saccharomyces cerevisiae. J. Biol. Chem. **271**, 18413–18422

13 Oh, C.-S., Toke, D. A., Mandala, S. and Martin, C. E. (1997) ELO2 and ELO3, homologues of the Saccharomyces cerevisiae ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation. J. Biol. Chem. **272**, 17376–17384

14 Knutzon, D. S., Thurmond, J. M., Huang, Y.-S., Chaudhary, S., Bobik, Jr, E. G., Chan, G. M., Kirchner, S. J. and Mukerji, P. (1998) Identification of Δ5-desaturase from *Mortierella alpina* by heterologous expression in bakers' yeast and canola. J. Biol. Chem. **273**, 29360–29366

15 Leonard, A. E., Kelder, B., Bobok, E. G., Chuang, L.-T., Parker-Barnes, J. M., Thurmond, J. M., Kroeger, P. E., Kopchick, J. J., Huang, Y.-S. and Mukerji, P. (2000). cDNA cloning and characterization of human Δ5-desaturase involved in the biosynthesis of arachidonic acid. Biochem. J. **347**, 719–724

16 Shanklin, J., Whittle, E. and Fox, B. G. (1998) Eight histidine residues are catalytically essential in a membrane-associated iron enzyme, stearoyl-CoA desaturase, and are conserved in alkane hydroxylase and xylene monooxygenase. Biochemistry **33**, 12787–12794

17 Hsu, M. H., Chirala, S. S. and Wakil, S. J. (1996) Human fatty-acid synthase gene. Evidence for the presence of two promoters and their functional interaction. J. Biol. Chem. **271**, 13584–13592

18 Dittrich, F., Zajonc, D., Huhne, K., Hoja, U., Ekici, A., Greiner, E., Klein, H., Hofmann, J., Bessoule, J. J., Sperling, P. and Schweizer, E. (1998) Fatty acid elongation in yeast – biochemical characteristics of the enzyme system and isolation of elongation-defective mutants. Eur. J. Biochem. **252**, 477–485

19 Wallis, J. G. and Browse, J. (1999) The Delta8-desaturase of Euglena gracilis: an alternate pathway for synthesis of 20-carbon polyunsaturated fatty acids. Arch. Biochem. Biophys. **365**, 307–316

20 Albert, D. H., Rhamy, R. K. and Coniglio, J. G. (1979) Desaturation of eicosa-11,14-dienoic acid in human testes. Lipids **14**, 498–500

21 Cook, H. W., Byers, D. M., Palmer, F. B., Spence, M. W., Rakoff, H., Duval, S. M. and Emken, E. A. (1991) Alternate pathways in the desaturation and chain elongation of linolenic acid, 18:3(n-3), in cultured glioma cells. J. Lipid. Res. **32**, 1265–1273

22 Grammatikos, S. I., Subbaiah, P. V., Victor, T. A. and Miller, W. M. (1994) n-3 and n-6 fatty acid processing and growth effects in neoplastic and non-cancerous human mammary epithelial cell lines. Br. J. Cancer **70**, 219–227

23 Dhopeshwarkar, G. A. and Subramanian, C. (1976) Intracranial conversion of linoleic acid to arachidonic acid: evidence for lack of delta8 desaturase in the brain. J. Neurochem. **26**, 1175–1179

24 Takayasu, K., Okuda, K. and Yoshikawa, I. (1970) Fatty acid composition of human and rat adrenal lipids: occurrence of omega 6 docosatrienoic acid in human adrenal cholesterol ester. Lipids **5**, 743–750

25 Garcia, P. T. and Holman, R. T. (1965) Competitive inhibitions in the metabolism of polyunsaturated fatty acids studied via the composition of phospholipids, triglycerides and cholesteryl esters of rat tissues. J. Am. Oil Chem. Soc. **42**, 1137–1141

26 The MHC Sequencing Consortium (1999) Complete sequence and gene map of a human major histocompatibility complex. Nature (London) **401**, 921–923

27 Cox, A., Camp, N. J., Cannings, C., di Giovine, F. S., Dale, M., Worthington, J., John, S., Ollier, W. E., Silman, A. J. and Duff, G. W. (1999) Combined sib-TDT and TDT provide evidence for linkage of the interleukin-1 gene cluster to erosive rheumatoid arthritis. Hum. Mol. Genet. **8**, 1707–1713

28 Gardner, R. J., Mungall, A. J., Dunham, I., Barber, J. C., Shield, J. P., Temple, I. K. and Robinson, D. O. (1999) Localisation of a gene for transient neonatal diabetes mellitus to an 18.72 cR3000 (approximately 5.4 Mb) interval on chromosome 6q. J. Med. Genet. **36**, 192–196

29 Olynyk, J. K., Cullen, D. J., Aquilia, S., Rossi, E., Summerville, L. and Powell, L. W. (1999) A population-based study of the clinical expression of the hemochromatosis gene. N. Engl. J. Med. **341**, 718–724

30 Lindholm, E., Ekholm, B., Balciuniene, J., Johansson, G., Castensson, A., Koisti, M., Nylander, P. O., Pettersson, U., Adolfsson, R. and Jazin, E. (1999) Linkage analysis of a large Swedish kindred provides further support for a susceptibility locus for schizophrenia on chromosome 6p23. Am. J. Med. Genet. **88**, 369–377

31 Delgado-Escueta, A. V., Medina, M. T., Serratosa, J. M., Castroviejo, I. P., Gee, M. N., Weissbecker, K., Westling, B. W., Fong, C. Y., Alonso, M. E., Cordova, S. et al. (1999) Mapping and positional cloning of common idiopathic generalized epilepsies: juvenile myoclonus epilepsy and childhood absence epilepsy. Adv. Neurol. **79**, 351–374

32 Oturai, A., Larsen, F., Ryder, L. P., Madsen, H. O., Hillert, J., Fredrikson, S., Sandberg-Wollheim, M., Laaksonen, M., Koch-Henriksen, N., Sawcer, S. et al. (1999) Linkage and association analysis of susceptibility regions on chromosomes 5 and 6 in 106 Scandinavian sibling pair families with multiple sclerosis. Ann. Neurol. **46**, 612–616

33 Davies, A. F., Mirza, G., Flinter, F. and Ragoussis, J. (1999) An interstitial deletion of 6p24-p25 proximal to the FKHL7 locus and including AP-2alpha that affects anterior eye chamber development. J. Med. Genet. **36**, 708–710

34 Kosel, S., Hofhaus, G., Maassen, A., Vieregge, P. and Graeber, M. B. (1999) Role of mitochondria in Parkinson disease. Biol. Chem. **380**, 865–870

35 Balendran, N., Clough, R. L., Arguello, J. R., Barber, R., Veal, C., Jones, A. B., Rosbotham, J. L., Little, A. M., Madrigal, A., Barker, J. N. et al. (1999) Characterization of the major susceptibility region for psoriasis at chromosome 6p21. J. Invest. Dermatol. **113**, 322–328

36 Hampe, J., Shaw, S. H., Saiz, R., Leysens, N., Lantermann, A., Mascheretti, S., Lynch, N. J., MacPherson, A. J., Bridger, S., van Deventer, S. et al. (1999) Linkage of inflammatory bowel disease to human chromosome 6p. Am. J. Hum. Genet. **65**, 1647–1655

37 Uauy, R., Peirano, P., Hoffman, D., Mena, P., Birch, D. and Birch, E. (1996) Role of essential fatty acids in the function of the developing nervous system. Lipids Suppl. S167–S176

38 Simopoulos, A. P. (1999) Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr. **70**, 560S–569S


Received 27 April 2000/22 June 2000; accepted 5 July 2000