Novel human DNA sequences that encode the gene CYB5RP, a delta 6 fatty acid desaturase, are provided. Provided are genomic CYB5RP DNA as well as cDNA that encodes the CYB5RP protein. Also provided is CYB5RP protein encoded by the novel DNA sequences. Methods of expressing CYB5RP protein in recombinant systems are provided. Also provided are CYB5RP methods that identify activators and inhibitors of CYB5RP protein.
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TITLE OF THE INVENTION
DELTA 6 FATTY ACID DESATURASE

CROSS-REFERENCE TO RELATED APPLICATIONS
Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D
Not applicable.

REFERENCE TO MICROFICHE APPENDIX
Not applicable.

FIELD OF THE INVENTION
The present invention is directed to novel human DNA sequences encoding a delta 6 fatty acid desaturase, an enzyme involved in the synthesis of essential fatty acids.

BACKGROUND OF THE INVENTION
Essential fatty acids (EFAs) are polyunsaturated fatty acids that cannot be manufactured by mammals, yet are required for a number of important biochemical processes, and thus must be supplied in the diet. The most important dietary EFAs are linoleic acid and alpha-linolenic acid (ALA). These two EFAs undergo a number of biosynthetic reactions that convert them into various other EFAs. Figure 1 depicts the biosynthetic reactions involving the two groups of EFAs, the n-6 EFAs (linoleic acid derivatives) and the n-3 EFAs (ALA derivatives). EFAs are formed from linoleic acid and ALA by a series of alternating reactions involving the removal of two hydrogens coupled with the insertion of an additional double bond (desaturation) and the lengthening of the fatty acid chain by the addition of two carbons (chain elongation). The enzymes catalyzing the desaturations and elongations are thought to be the same for both groups of EFAs.

Among the more important unsaturated fatty acids are the delta 6 unsaturated fatty acids, which are involved in the maintenance of membrane structure and function, the regulation of cholesterol synthesis and transport, and the prevention
of water loss from the skin. Delta 6 unsaturated fatty acids also serve as precursors of the eicosanoids, including the prostaglandins and leukotrienes (Horrobin, 1992, Prog. Lipid Res. 31:163-194). The double bond at the 6 position of delta 6 unsaturated fatty acids is introduced by a class of enzymes known as delta 6 desaturases.

Deficiencies in linoleic acid and ALA derivatives have been associated with skin diseases, diabetic complications, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infection, and retinal dysfunction. For example, a deficiency in gamma-linolenic acid (GLA), which is produced from linoleic acid by the action of the enzyme delta 6 desaturase, can arise from the decreased activity of this enzyme that occurs in aging, stress, diabetes, eczema, and some infections, or from increased catabolism of GLA due to oxidation or rapid cell division, as occurs in inflammation or cancer. Clinical trials have demonstrated that dietary GLA supplementation can be effective in treating a number of conditions that are associated with GLA deficiency, e.g., atopic eczema, mastalgia, diabetic neuropathy, viral infections, and some forms of cancer (Horrobin, 1990, Rev. Contemp. Pharmacother. 1:1-45).

Delta 6 desaturase is an example of a fatty acid desaturase. Fatty acid desaturases are enzymes that introduce a double bond into the carbon chain of fatty acids. They play vital roles in the biosynthesis of polyunsaturated fatty acids, including the essential fatty acids. Fatty acid desaturases are present in soluble and membrane-associated forms and require electron donors (for example, cytochrome b5) for their functioning.

Delta 6 desaturases catalyze the rate-limiting steps in the biosyntheses of the linoleic and ALA group EFAs shown in Figure 1. End products of the linoleic acid pathway include the eicosanoids (prostaglandins and leukotrienes). The end product of the ALA pathway is docosahexaenoic acid (DHA), an important component of membranes in the vertebrate retina. DHA is highly specific for retina and represents more than 50% of the fatty acids in the rod outer segment (ROS). It appears that DHA is important in maintaining the normal structure and function of the retina (Anderson et al., 1992, Neurobiology of Essential Fatty Acids, Bazan et al., eds., Plenum Press, New York, pages 285-294). Increased dietary consumption of DHA and its precursor, eicosapentaenoic acid, from seal meat and fish has been

Certain delta 6 desaturases have been cloned from plants. For example, a delta 6 desaturase has been cloned from borage (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216). This delta 6 desaturase is unusual in that its cytochrome b5 electron donor is present as an N-terminal extension of the enzyme rather than being synthesized as a separate protein. The borage delta 6 desaturase has been shown to be functional, in that transfer of the cloned gene encoding it to tobacco results in the synthesis of high levels of GLA and octadecatetraenoic acid (OTA) in the transgenic tobacco leaves. GLA and OTA are the products of delta 6 desaturase activity on linoleic acid and ALA, respectively.

Based on its hydrophathy profile, the borage delta 6 desaturase appears to be a membrane-bound protein. Examination of the amino acid sequence of the borage enzyme, as well as the amino acid sequences of membrane-bound desaturases from a wide variety of organisms, has revealed three regions of conserved short motifs containing histidine residues (HX(3 or 4)H, HX(2 or 3)HH, and HX(2 or 3)HH) having a conserved spacing from each other (Shanklin et al., Biochemistry, 1994, 33:12787-12794).

A DNA sequence has been isolated from sunflower embryos that, judging from its sequence, appears to encode a delta 6 desaturase having a cytochrome b5-like moiety fused to its N-terminus (Sperling et al., 1995, Eur. J. Biochem. 232:798-805).

SUMMARY OF THE INVENTION

The present invention is directed to novel human DNA sequences that encode a delta 6 fatty acid desaturase, cytochrome b5-related protein (CYB5RP). The present invention includes genomic CYB5RP DNA as well as cDNA that encodes the CYB5RP protein. The genomic CYB5RP DNA is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. The cDNA encoding CYB5RP protein is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:2. Also provided is CYB5RP protein encoded by the novel DNA sequences. The CYB5RP protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:3.
Methods of expressing CYB5RP protein in recombinant systems are provided. Also provided are methods of producing delta 6 unsaturated fatty acids using DNA encoding CYB5RP or using CYB5RP protein.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the enzymatic conversions involved in the linoleic acid (n-3) and alpha-linolenic acid (n-6) pathways of essential fatty acid synthesis.

Figure 2A-G shows the genomic DNA sequence of the CYB5RP gene (SEQ.ID.NO.:1). Underlined nucleotides in capitals represent exons. The start ATG codon at position 544 in exon 1 and the stop TGA codon at position 18,103 in exon 12 are shown in bold. The putative polyadenylation signal ATTAAA located approximately 20 base pairs upstream of the polyA tail is shown in bold italics (position 18,373 in exon 12). DNA sequence upstream of exon 1 represents a putative promoter region of the CYB5RP gene, as indicated by the presence of the TATA box at position 353 (underlined bold).

Figure 3A-C shows the cDNA sequence (SEQ.ID.NO.:2) and the amino acid sequence (SEQ.ID.NO.:3) of CYB5RP. The region encompassing amino acids 1-102 represents the cytochrome b5 domain. The region encompassing amino acids 182-186 represents HIS BOX 1. The region encompassing amino acids 219-223 represents HIS BOX 2. The region encompassing amino acids 383-387 represents HIS BOX 3.

Figure 4 shows a portion of the cDNA sequence (SEQ.ID.NO.:4) and a portion of the amino acid sequence (SEQ.ID.NO.:5) of mouse CYB5RP.

Figure 5A shows a Kyte-Doolittle hydropathy plot of CYB5RP.

Figure 5B shows the proposed membrane topology of CYB5RP based on its hydropathy plot. This membrane topology is similar to that proposed for other membrane-bound fatty acid desaturases (Shanklin et al., Biochemistry, 1994, 33:12787-12794). The amino acids shown in Figure 5B are portions of (SEQ.ID.NO.:3).

Figure 6 shows the output of the Profilescan program from the Wisconsin GCG package. The upper amino acid sequence is from CYB5RP (positions 31-78 of SEQ. ID. NO.3). The lower amino acid sequence is positions 1-48 of the cytochrome b5 profile (SEQ. ID. NO.:6.). The output shows that CYB5RP
contains a profile typical for the heme-binding domain of the cytochrome b5 protein family. Importantly, the region of identity includes the invariant HPGG motif, where histidine represents a heme axial ligand for iron.

Figure 7A and B show the results of BlastP searches of the GenBank database using the full-length CYB5RP amino acid sequence as the query. Figure 7A shows the hit with highest homology, a hypothetical protein from sunflower. The sunflower protein and CYB5RP share three His boxes (boxed) in which the spacing between the His boxes is conserved. Also boxed is the HPGG motif typical for the heme-binding domain of the cytochrome b5 protein family. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.:3. The lower amino acid sequences shown are portions of the amino acid sequence of the hypothetical protein from sunflower (Sperling et al., 1995, Eur. J. Biochem. 232:798-805). The sequence shown as positions 348-432 is SEQ. ID. NO.:7. The sequence shown as positions 22-74 is SEQ. ID. NO.:8. The sequence shown as positions 152-227 is SEQ. ID. NO.:9.

Figure 7B shows the hit with the second highest homology, a delta 6 desaturase from Borago officinalis (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216). The Borago protein and CYB5RP also share three His boxes with conserved spacing, as well as the HPGG motif. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.:3. The lower amino acid sequences shown are portions of the amino acid sequence of the Borago delta 6 desaturase. The sequence shown as positions 338-424 is SEQ. ID. NO.:10. The sequence shown as positions 12-64 is SEQ. ID. NO.:11. The sequence shown as positions 153-220 is SEQ. ID. NO.:12.

Figure 8 shows additional results of BlastP searches of the GenBank database using the CYB5RP protein as the query. Figure 8 shows the amino acid alignment between the CYB5RP protein and a delta 6 desaturase from Synechocystis sp. (strain pcc 6803) performed by the BlastP program. The Synechocystis delta 6 desaturase and CYB5RP share three His boxes, two of which are shown in Figure 8 (boxed). In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The CYB5RP
sequence shown is a portion of SEQ. ID. NO.3. The *Synechocystis* sequence shown is
SEQ. ID. NO:13.

Figure 9A shows the expression pattern of the CYB5RP gene in 9
human tissues, as determined by RT-PCR amplification with 21 cycles. Expression is
detected in human retina, kidney, pancreas, placenta, and brain. Figure 9B shows the
results of the analogous experiments performed with 25 cycles of amplification.
Expression of the CYB5RP gene is seen in all the human tissues studied.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably
95%, more preferably 99%, and even more preferably 99.9%, free of other proteins.
Thus, a CYB5RP protein preparation that is substantially free from other proteins will
contain, as a percent of its total protein, no more than 10%, preferably no more than
5%, more preferably no more than 1%, and even more preferably no more than 0.1%,
of non-CYB5RP proteins. Whether a given CYB5RP protein preparation is
substantially free from other proteins can be determined by such conventional
techniques of assessing protein purity as, e.g., sodium dodecyl sulfate polyacrylamide
gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, e.g.,
silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least 90%,
preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other
nucleic acids. Thus, a CYB5RP DNA preparation that is substantially free from other
nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%,
preferably no more than 5%, more preferably no more than 1%, and even more
preferably no more than 0.1%, of non-CYB5RP nucleic acids. Whether a given
CYB5RP DNA preparation is substantially free from other nucleic acids can be
determined by such conventional techniques of assessing nucleic acid purity as, e.g.,
agarose gel electrophoresis combined with appropriate staining methods, e.g.,
ethridium bromide staining, or by sequencing.

"Substantially the same biological activity as CYB5RP" means being
able to introduce a double bond into the 6 position of linoleic acid under conditions in
which CYB5RP is able to introduce a double bond into the 6 position of linoleic acid.
A “conservative amino acid substitution” refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

The present invention relates to the identification and cloning of cytochrome b5-related protein (CYB5RP), a gene which encodes a human delta 6 fatty acid desaturase. The gene is present on PAC clones 759J12, 756B3, 519O13, and 466A11 from an area of human chromosome 11q12 that has been shown to contain a gene related to Best’s macular dystrophy (Cooper et al., 1997, Genomics 41:185-192; Stöhr et al., 1997, Genome Res. 8:48-56; Graff et al., 1997, Hum. Genet. 101: 263-279). This linkage between the chromosomal location of the CYB5RP gene and the location of the gene related to Best’s macular dystrophy can be used diagnostically by identifying restriction fragment length polymorphisms (RFLPs) in the vicinity of the CYB5RP gene, e.g., in SEQ.ID.NO.:1. Such RFLPs will be associated with the Best’s macular dystrophy gene and thus can be used to identify individuals carrying disease-causing forms of the Best’s macular dystrophy gene.

CYB5RP was identified as an EST hit in sequence scanning data from PAC clones from human chromosome 11q12. In addition, a full length cDNA of CYB5RP was recovered from a human retina cDNA library. The genomic region of CYB5RP has been sequenced and the exon/intron organization of CYB5RP has been determined. The CYB5RP gene has 12 exons. The promoter region of CYB5RP was identified upstream of the 5’ UTR by detecting consensus elements required for eukaryotic transcription. The expression pattern of CYB5RP was determined by RT-PCR analysis in 9 human tissues. The CYB5RP gene is expressed predominantly in human retina, kidney, pancreas, and placenta; lower levels of expression are also detected in brain, heart, lung, liver, and skeletal muscle. Bioinformatic analysis revealed significant homology to a group of plant and bacterial fatty acid desaturases. All of the typical amino acid motifs present in these fatty acid desaturases are also present in CYB5RP. Kyte-Doolittle algorithm analysis predicts a transmembrane organization typical of fatty acid desaturases for CYB5RP (see Figure 5). CYB5RP is
unusual in that it contains a cytochrome b5 region in its N terminus. While many fatty acid desaturases utilize cytochrome b5 as an electron donor, most have not incorporated this cytochrome as part of their polypeptide chain.

That CYB5RP is a fatty acid desaturase is shown by the following evidence:

(1) CYB5RP possesses significant homology to a group of plant and microbial fatty acid desaturases;
(2) Like other fatty acid desaturases, CYB5RP has three conserved histidine boxes, with correct spacing between the boxes; and
(3) The predicted membrane topology of CYB5RP is similar to that of known fatty acid desaturases.

That CYB5RP is a delta 6 fatty acid desaturase is shown by the following evidence:

(1) CYB5RP contains a cytochrome b5-like moiety fused to its N-terminus. The only two fatty acid desaturases that contain cytochrome b5-like moiety fused to their N-termini are known or suspected to be delta 6 desaturases.
(2) The only two plant desaturases that are known or suspected to introduce a double bond in the 6 position have an atypical His box 3 (QI/LEHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.
(3) The only bacterial desaturase that is known to introduce a double bond in the 6 position has an atypical His box 3 (QVTHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.

CYB5RP is a target for the development of drugs for the treatment of disorders of lipid metabolism and for the treatment of conditions that require the modulation of the biosynthesis of prostaglandins and leukotrienes (asthma, pain, etc.). CYB5RP is also a target for the development of drugs for use in treating skin diseases, diabetic complications, reproductive disorders, including breast pain and premenstrual syndrome, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infections, and various forms of retinal degeneration, including age-related macular degeneration.

CYB5RP is homologous to a delta 6 desaturase from Borago officinalis (see Figure 7B). Both CYB5RP and this Borago delta 6 desaturase, unlike desaturases from higher plants, are unusual in containing a cytochrome b5-like
domain fused to their N-termini (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216; hereinafter "Sayanova"). The Borago desaturase has been expressed in transgenic tobacco, resulting in high levels of delta 6 desaturated fatty acids in the transgenic tobacco leaves, including high levels of $\gamma$-linolenic acid (GLA) (Sayanova).

Given the medical importance of GLA, Sayanova proposed that transgenic plants, expressing the Borago delta 6 desaturase, would be valuable as sources of GLA. Similarly, CYB5RP, expressed in transgenic plants, is expected to provide a valuable source of GLA.

The present invention provides DNA encoding CYB5RP that is substantially free from other nucleic acids. The present invention also provides recombinant DNA molecules encoding CYB5RP. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequence shown in Figure 2 as SEQ.ID.NO.:1. Analysis of SEQ.ID.NO.:1 revealed that this genomic sequence defines a gene having 12 exons. These exons collectively have an open reading frame that encodes a protein of 445 amino acids. When an alternatively spliced exon 8 is used, a CYB5RP protein of 433 amino acids, lacking amino acids 317-328, is produced. Thus, the present invention includes two cDNA molecules, encoding two forms of CYB5RP protein, that are substantially free from other nucleic acids. The first cDNA is shown in Figure 3 and has the nucleotide sequence SEQ.ID.NO.:2. The second cDNA is identical to the first, except that it does not contain the nucleotides at positions 1,019-1,054.

The present invention includes DNA molecules substantially free from other nucleic acids comprising the coding region of SEQ.ID.NO.:2. Accordingly, the present invention includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2. The present invention also includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2, except that the nucleotides at positions 1,019-1,054 are missing. Also included in the present invention are recombinant DNA molecules having a nucleotide sequence comprising positions 71-1,405 of SEQ.ID.NO.:2 and recombinant DNA molecules having a nucleotide sequence comprising positions 71-1,405 of SEQ.ID.NO.:2 with the exception that positions 1,019-1,054 are missing.
The novel DNA sequences of the present invention encoding CYB5RP, in whole or in part, can be linked with other DNA sequences, i.e., DNA sequences to which CYB5RP is not naturally linked, to form “recombinant DNA molecules” encoding CYB5RP. Such other sequences can include DNA sequences that control transcription or translation such as, e.g., translation initiation sequences, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide “tag” such as, e.g., a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NO.s:1 or 2 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows:

Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt’s solution, and 100 μg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of 32-P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt’s solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the
construction of synthetic DNA that encodes the CYB5RP protein where the nuclotide sequence of the synthetic DNA differs significantly from the nuclotide sequence of SEQ.ID.NO.:2, but still encodes the same CYB5RP protein shown as SEQ.ID.NO.:3. Such synthetic DNAs are intended to be within the scope of the present invention. Also with the scope of the present invention are synthetic DNAs that encode a CYB5RP protein lacking amino acids 317-328 of SEQ.ID.NO.:3.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding CYB5RP protein. Such recombinant host cells can be cultured under suitable conditions to produce CYB5RP protein. An expression vector containing DNA encoding CYB5RP protein can be used for expression of CYB5RP protein in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as E. coli, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, plant cells such as tobacco, and insect cells including but not limited to Drosophila and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of CYB5RP protein and which are commercially available, include but are not limited to, L cells L-M(TK-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

A variety of mammalian expression vectors can be used to express recombinant CYB5RP in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, CYB5RP can be purified by conventional techniques to a level that is substantially free from other proteins. A description of vectors that can be used to express CYB5RP can be found in, e.g., Goeddel, ed., 1990, Meth. Enzymol. vol. 185 or Perbal, 1988, A Practical Guide to Molecular Cloning, John Wiley and Sons, Inc.
The present invention includes CYB5RP protein substantially free from other proteins. The amino acid sequence of the full-length CYB5RP protein is shown in Figure 3 as SEQ.ID.NO.:3. Thus, the present invention includes CYB5RP protein substantially free from other proteins having the amino acid sequence SEQ.ID.NO.:3. Also included in the present invention is a CYB5RP protein that is produced from an alternatively spliced CYB5RP mRNA where the protein has the amino acid sequence of SEQ.ID.NO.:3 with the exception that amino acids 317-328 are missing.

As with many proteins, it is possible to modify many of the amino acids of CYB5RP and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified CYB5RP proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as CYB5RP. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., Molecular Biology of the Gene, Watson et al., 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as CYB5RP. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the His boxes of CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the sunflower protein depicted in Figure 1 of Sperling et al., 1995, Eur. J. Biochem. 232:798-805 when these two proteins are aligned by BLASTP analysis. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the
CCCTCTACCCCTGTCATCTAGGC (SEQ.ID.NO.:15)

One of skill in the art would recognize that many other primer pairs based upon SEQ.ID.NO.:2 would also be suitable.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl₂, 200 μM for each dNTP, 50 mM KCl, 0.2 μM for each primer, 10 ng of DNA template, 0.05 units/µl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael et al., eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding CYB5RP can be isolated would be Human Retina 5′-stretch cDNA library in lambda gt10 or lambda gt11 vectors (catalog numbers HL1143a and HL1132b, Clontech, Palo Alto, CA). The primary clones of such a library can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of either 445 amino acids (SEQ.ID.NO.:3) or an open reading frame of 433 amino acids (SEQ.ID.NO.:3 lacking the amino acids at positions 317-328) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA). CYB5RP protein can then be produced by transferring an expression vector encoding CYB5RP or portions thereof into a suitable host cell and growing the host cell under appropriate conditions. CYB5RP protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding CYB5RP can be isolated from a cDNA library using as a probe oligonucleotides specific for CYB5RP and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described
in, e.g., Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual; Cold
Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985,
Oligonucleotides that are specific for CYB5RP and that can be used to screen cDNA
libraries can be readily designed based upon the cDNA sequence of CYB5RP shown
in SEQ.ID.NO.:2 and can be synthesized by methods well-known in the art.

Genomic clones containing the CYB5RP gene can be obtained from
commercially available human PAC or BAC libraries available from Research
Genetics, Huntsville, AL. PAC clones containing the CYB5RP gene (e.g., PAC
clones 759J12, 756B3, 519O13, and 466A11) are commercially available from
Research Genetics, Huntsville, AL. (Catalog number for individual PAC clones is
RPC1.C). Alternatively, one may prepare genomic libraries, especially in P1 artificial
chromosome vectors, from which genomic clones containing the CYB5RP can be
isolated, using probes based upon the CYB5RP sequences disclosed herein. Methods
of preparing such libraries are known in the art (Ioannou et al., 1994, Nature Genet.
6:84-89).

The present invention also provides oligonucleotide probes, based
upon SEQ.ID.NO.:2 that can be used to determine the level of CYB5RP RNA in a
sample. In particular, the present invention includes DNA oligonucleotides
comprising at least 18 contiguous nucleotides of SEQ.ID.NO.:2. Also provided by
the present invention are corresponding RNA oligonucleotides. The DNA or RNA
oligonucleotide probes can be packaged in kits.

In addition to the utilities described above, the present invention makes
possible the recombinant expression of the CYB5RP protein in various cell types. In
particular, it is advantageous to recombinantly express CYB5RP in plant cells. Such
expression in plant cells provides a method for the production of high levels of
valuable EFAs such as GLA and OTA in the recombinant plant cells. An example of
such recombinant expression of a delta 6 fatty acid desaturase, in that case from
borage, is described in Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-
4216 (Sayanova). The recombinant expression of the borage delta 6 desaturase led to
the production of high levels of GLA and OTA in the leaves of the tobacco plants in
which it was expressed. The procedures described in Sayanova can be easily adapted
to express CYB5RP in tobacco, thus providing an additional, useful way to produce
large amounts of valuable EFAs. Known methods of recombinantly expressing genes in other plant species beside tobacco can be used to express CYB5RP in those other species.

The present invention also makes possible the development of assays which measure the biological activity of the CYB5RP protein. Such assays using recombinantly expressed CYB5RP protein are especially of interest.

Assays for CYB5RP protein activity can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of CYB5RP protein. Such identified compounds can serve as “leads” for the development of pharmaceuticals that can be used to modulate the activity of CYB5RP in patients suffering from conditions where that activity is abnormal, e.g., skin diseases, diabetic complications, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infection, and retinal dysfunction such as macular degeneration.

Such assays may comprise:
(a) recombinantly expressing CYB5RP protein in a host cell;
(b) measuring the biological activity of the recombinantly expressed CYB5RP protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CYB5RP protein;

where a change in the biological activity of the recombinantly expressed CYB5RP protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CYB5RP protein.

In particular embodiments, the biological activity of the recombinantly expressed CYB5RP protein is the ability to introduce a double bond into the 6 position of linoleic acid or alpha-linoleic acid.

In some embodiments, it may be advantageous to insert additional steps between steps (a) and (b). Such additional steps might include lysing the host cell and fractionating its contents in order to partially purify the recombinantly expressed CYB5RP, thus facilitating exposure of the recombinantly expressed CYB5RP to the substance as well as to any substrate used in the assay.

The present invention includes activators and inhibitors identified by the methods described herein as well as pharmaceutical compositions comprising...
such activators and inhibitors. The activators and inhibitors are generally combined with pharmaceutically acceptable carriers before use to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing activators or inhibitors and carriers can be found in Remington’s Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the activator or inhibitor.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where CYB5RP activity is abnormal. The effective amount can vary according to a variety of factors such as the individual’s condition, weight, sex and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection.

Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route
of administration; the renal, hepatic and cardiovascular function of the patient; and
the particular composition thereof employed. A physician or veterinarian of ordinary
skill can readily determine and prescribe the effective amount of the composition
required to prevent, counter or arrest the progress of the condition. Optimal precision
in achieving concentrations of composition within the range that yields efficacy
without toxicity requires a regimen based on the kinetics of the composition's
availability to target sites. This involves a consideration of the distribution,
equilibrium, and elimination of a composition.

The present invention also includes antibodies to the CYB5RP protein.

Such antibodies may be polyclonal antibodies or monoclonal antibodies. The
antibodies of the present invention are raised against the entire CYB5RP protein or
against suitable antigenic fragments of the protein that are coupled to suitable carriers,
e.g., serum albumin or keyhole limpet hemocyanin, by methods well known in the art.
Methods of identifying suitable antigenic fragments of a protein are known in the art.
See, e.g., Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and
Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-
186.

For the production of polyclonal antibodies, CYB5RP protein or an
antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an
appropriate non-human host animal such as, e.g., rabbits, sheep, goats, rats, mice.
The animals are bled periodically and sera obtained are tested for the presence of
antibodies to the injected antigen. The injections can be intramuscular,
intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, CYB5RP protein or an
antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-
human host animal as above for the production of polyclonal antibodies. In the case
of monoclonal antibodies, the animal is generally a mouse. The animal’s spleen cells
are then immortalized, often by fusion with a myeloma cell, as described in Kohler &
Milstein, 1975, Nature 256:495-497. For a fuller description of the production of
monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds.,

Gene therapy may be used to introduce CYB5RP polypeptides into the
cells of target organs, e.g., the pigmented epithelium of the retina or other parts of the
retina. Nucleotides encoding CYB5RP polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding CYB5RP polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with CYB5RP polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate CYB5RP activity.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.
WHAT IS CLAIMED:

1. A recombinant DNA molecule encoding a polypeptide having the amino acid sequence of SEQ.ID.NO.:3.

2. A recombinant DNA molecule comprising a nucleotide sequence selected from the group consisting of:
   SEQ.ID.NO.:1;
   SEQ.ID.NO.:2;
   SEQ.ID.NO.:2 lacking positions 1,019-1,054;
   positions 71-1,405 of SEQ.ID.NO.:2; and
   positions 71-1,405 of SEQ.ID.NO.:2 lacking positions 1,019-1,054.

3. A DNA molecule that hybridizes under stringent conditions to the DNA molecule of claim 2.

4. An expression vector comprising the DNA of claim 1.

5. A recombinant host cell comprising the DNA of claim 1.

6. A CYB5RP protein, substantially free from other proteins, having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:3 and SEQ.ID.NO.:3 lacking positions 317-328.

7. The CYB5RP protein of claim 6 containing a single amino acid substitution.

8. The CYB5RP protein of claim 7 where the substitution is a conservative substitution.

9. The CYB5RP protein of claim 6 containing amino acid substitutions where the substitutions do not occur in positions where the amino acid
present in CYB5RP at those positions is also present in the corresponding position in
the delta 6 desaturase from sunflower when CYB5RP and the delta 6 desaturase from
sunflower are aligned by BLASTP analysis or where the substitutions do not occur in
positions where the amino acid present in CYB5RP at those positions is also present
in the corresponding position in the delta 6 desaturase from Synechocystis when
CYB5RP and the delta 6 desaturase from Synechocystis are aligned by BLASTP
analysis or where the substitutions do not occur in positions where the amino acid
present in CYB5RP at those positions is also present in the corresponding position in
the delta 6 desaturase from borage when CYB5RP and the delta 6 desaturase from
borage are aligned by BLASTP analysis.

10. An antibody that binds specifically to the CYB5RP protein of
claim 6.

11. A DNA or RNA oligonucleotide probe comprising at least 18
contiguous nucleotides of at least one of the sequences of claim 2.

12. A method for determining whether a substance is an activator
or an inhibitor of CYB5RP protein comprising:
(a) recombinantly expressing the CYB5RP protein of claim 6 in a
host cell;
(b) measuring the biological activity of the recombinantly
expressed CYB5RP protein in the presence and in the absence of a substance
suspected of being an activator or an inhibitor of CYB5RP protein;
where a change in the biological activity of the recombinantly
expressed CYB5RP protein in the presence as compared to the absence of the
substance indicates that the substance is an activator or an inhibitor of CYB5RP
protein.

13. The method of claim 12 where the biological activity of
CYB5RP protein is the ability to introduce a double bond into the 6 position of
linoleic acid.
14.  A pharmaceutical composition comprising an activator or an inhibitor of CYB5RP.

15.  A method of treating macular degeneration comprising administering to a patient an effective amount of the pharmaceutical composition of claim 14.
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**FIG. 1**
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**FIG.2D**
| 10701 | cactccagct atacacagaa atgcgcagaa tttgggattta ttcatttttat  |
| 10751 | ttatgtgttt tctttttttt taaaatatag gacaaggtct cactatgtgg  |
| 10801 | cccaggtcgg tcttgactctg tgcgtcccaa gcacatgctg tgccttgccc  |
| 10851 | ttgccagctg atggaggtac agatgtgagc cacatgctct gcgcctatttt  |
| 10901 | cagcttctctg aggcttggttc ttgatttttg gactgtctgc tatttttttt  |
| 10951 | ggcttagctg tctttttttt taaaatatag gacaaggtct cactatgtgg  |
| 11001 | ttaaaragcag cttggtccacc tggggtcagg atgtggtgag cagcttctct  |
| 11051 | gctctctctct gcctctctct gcctctctct gcctctctct gcctctctct  |
| 11101 | ctctctctct gcctctctct gcctctctct gcctctctct gcctctctct  |
| 11151 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11201 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11251 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11301 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11351 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11401 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11451 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11501 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11551 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11601 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11651 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11701 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |
| 11751 | ttaggtgtga tggaggtgta tggaggtgta tggaggtgta tggaggtgta  |

**FIG.2E**
FIG. 3B
FIG. 3C
12/19

1 GTACAGCGGCAATGGGGGTGTGGGAGGCCCGAGGGGACTCGGGCGG  50
   M G G V G E P G G G L G P  13

51 CCGGAGGGGCCCGACCCGCTGGGGCGCCCTACCATTCTCGCTGGGA  100
14 REGPAPLGLAPLPFPWRE  30

101 GCAGATCCGCCACACGAGCCCTACCAGGGCACAAGTGCTGTCATCGAGC  150
31 QIRQHDLPGDKWLVIER  47

151 GCCGTGTCTACGACATCAGCCGCTGGGCACAGCGCGACCCAGGGGTAGC  200
48 RVYDISRWAQRHPGGS  63

201 CGCATCATCGGCGACCAACCGG  220
64 RIIIGHH  69

FIG. 4
PROFILESCAN of: CYB5rp_correct_protein check: 5714 from: 1 to: 445

GETSEQ from bmd, December 2, 1997 14:20.

Compare to profile library: GenRunData:profilescan.fil

Profile: profiledir:cytochrome_b5.prf
  Gap weight: 4.50  Gap Length weight: 0.05
  Ave match: 0.27  Ave mismatch : -0.21
(Peptide) PROFILEMAKE v4.40 of: 0191.Ms12{+} Length: 48
  Sequences: 24  MaxScore: 27.58  December 2, 1992 00:07
This profile is derived from PROSITE release 10.0 and has been tested
by a database search against SWISS-PROT release 26.0. A comparison
of the SWISS-PROT annotation and the results of the database search follows.
For further information about this motif, consult the . . .

Profile: profiledir:cytochrome_b5.prf alignment: 1

Quality: 20.77  Gaps: 0
  Ratio: 0.43  Length: 48
  Normalized quality: 2.91

S  31 HDQPDKWLVIIERRylvISRWAQRHPGSGRNLICHGCAEDATDAFRAFH 78
  [: ..: ||||. -|||:: | |||| . -||.:: ||:: |
P  1 HNDGETWLVNGQVYDITKFLEEHPGPDVIMEAAGTDATEEFEAIH 48

**********************************************************************
*Cytochrome b5 family, heme-binding domain signature*
**********************************************************************

**FIG. 6**
pir:s68358  hypothetical protein - common sunflower
Length = 458

Score = 169 (79.4 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
Identities = 31/85 (36%), Positives = 49/85 (57%)  His box 3

Query:  348 IGHEKHRDWNSSQAATCNEPSLFTNWFSCHLNFQIEHHFPRMPRHNRYSRVALPKSL 407
        +G K  +W Q  T ++ S ++WF G L FQIEHHFPR+PR ++P+ + L
Sbjct:  348 VGGPKGDWMEKQTRTGIDIACSSMDFWFGGQLFQLEHHFPRPRCHLRSISPICREL 407

Query:  408 CAKHGLSEYVKPFLTLAVDIVRSKL 432
        C K+ L Y  F A V  +++H++
Sbjct:  408 CKKYNLPYVSLSFYDANVTLKTLR 432

Score = 133 (62.5 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
Identities = 21/53 (39%), Positives = 35/53 (66%)  HPGG motif

Query:  26 EQIRAHDOQPKWLVIERVYDISRWAQRRHPCGSLIGHHGAEADTAFRAFH 78
        +++ H+ P  D W+ I ++VY+++ WA+ HPGG  +++  +D TDAF AFH
Sbjct:  22 KELLKHNPMNLWISILGKVYNTFELAPCQDAPLNLAGQDVTDARFIACH 74

Score = 118 (55.5 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
Identities = 25/76 (32%), Positives = 34/76 (44%)  His box 1

Query:  165 LAAFLALISDAQWSCLQHDLGHAISTFKSSWNNHVAQKFVMGQLKFSAHWNFRFQHEA 224
        L+ IL ++ Q  L HD GH++ WN A F+ + G S WW + H HH
Sbjct:  152 LSGAILGLAWMQIAYLHGDACHYQMMATRGWKNKFAGIFGNCITGSIAWWKTWMHNAHH 211

Query:  225 KPNIFHKDPDTVAPV 240
        N DPD+ P+
Sbjct:  212 ACNSLDYDPDLQHLP 227

Score = 34 (16.0 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
Identities = 7/14 (50%), Positives = 9/14 (64%)

FIG. 7A
gp:bou79010 1 PID:q2062403 Borago officinalis delta 6 desaturase mRNA, complete cds. (gb:U79010) (NID:2062402)
Length = 448

Score = 179 (84.1 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42
Identities = 34/87 (39%), Positives = 48/87 (55%) His box 3

Query: 348 IGHEKIRDWSSQLATCNVPESLFTNWFSGHNPIEJEHLPFRMPRHNYSRVMVPLKSL 407
+G K +W Q T ++ +WF G L QJEHEHLPF+MPR N +++P V L
Sbjct: 338 VGPGBKNNWFEKQDTGTLDDSCPWMDFWHCLQFQJEHLPKMPERNLKISPYVIEL 397

Score = 144 (67.7 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42
Identities = 23/53 (43%), Positives = 36/53 (67%) HPGG MOTIF

Query: 26 EQRRAHDQPGDKWLVIERRVYDISRWAA+HPGGSRLGIGHHGAEDMATDAFRAFH 78
++++ HD+PGD W+ I+ YD+S W + HPGGS + ++ TDAF AFH
Sbjct: 12 DELKNDKPGDLWISIQGKAYDVSOWKDHPGSFLKLQGQEVTDADFVAFH 64

Score = 105 (49.3 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42
Identities = 22/68 (32%), Positives = 28/68 (41%) His box 2

Query: 176 QSWCLCFLHDGHASIFKKSWNHVAQFKVWCMQGLKGFSAHWWNF+QFHHHAKPNIFHKDPDV 235
QS + HD GH + S N F L G S WW + H HH N DPD+
Sbjct: 153 QSCWICHGDCHMVVSRLNKFMGFAANCLSCGIGWKKWNINAHHIAACSNLEYDPDL 212

Score = 236 TVAPVFLL 243
p ++
Sbjct: 213 QVIPFLVW 220

FIG. 7B
\( \text{pir:s35157 Δelta(6)-desaturase - Synechocystis sp.} \)

Length = 359

Score = 126 (59.2 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09
Identities = 21/54 (38%), Positives = 33/54 (61%)

**His box 3**

Query: 372 FTNWSGHLNFOIEHLLFPWRHPHRHNSRVRPLVKSICAKHGLSEVCPKFLALV 425
        F NMF G LN Q+ HLF + +Y ++ ++K +C +G +Y+V P A+

Sbjct: 292 FWNWFCNGSLNHQTVHLLPNICHIHYPQLENIKIDVCQEGVYEKVPFTKAAI 345

Score = 36 (16.9 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09
Identities = 6/15 (40%), Positives = 8/15 (53%)

**His box 2**

Query: 209 GFSAHWNFRHFCOHH 223
        G S+ W +R +H+

Sbjct: 113 GLSSFLWRYRHNYLH 127

**FIG. 8**
1  2  3  4  5  6  7  8  9

3. Placenta  8. Pancreas
4. Lung  9. Retina
5. Liver

FIG. 9A
1. Heart
2. Brain
3. Placenta
4. Lung
5. Liver
6. Skeletal Muscle
7. Kidney
8. Pancreas
9. Retina

FIG. 9B
## INTERNATIONAL SEARCH REPORT

### A. CLASSIFICATION OF SUBJECT MATTER

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<th>US CL</th>
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<td>435/135, 189, 320.1, 452.3; 424/130.1; 536/23.2</td>
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According to International Patent Classification (IPC) or to both national classification and IPC.

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S.: 435/135, 189, 320.1, 452.3; 424/130.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

- Medline
- Search terms: CYB5RP, delta-6 fatty acid desaturase, human, or homo sapiens

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>Database GenBank, Accession AAC23396, submitted by LAMERDIN, JE, publicly available on 12 June 1998, see entire record.</td>
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<tr>
<td>X</td>
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<tr>
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<td>X</td>
<td>WO 98/39446 A2 (HUMAN GENOME SCIENCES, INC.) 11 September 1998, see entire document, especially SEQ ID No:63.</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

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<th>Special category of cited documents:</th>
<th>Document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</th>
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<td>&quot;B&quot;</td>
<td>Document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone.</td>
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Date of the actual completion of the international search: 24 FEBRUARY 2000

Date of mailing of the international search report: 15 MAR 2000

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
BRADLEY S. MARTIN
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*
B. FIELDS SEARCHED

Documentation other than minimum documentation that are included in the fields searched:

Because a CRF was not made available at the time of the search, Database GenBank Accession AF134404, which appears to encode the same desaturase as set forth in Figures 3A-C of the instant application, was searched against all available amino acid and nucleic acid databases.