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(54) Title: ELONGASE GENES AND USES THEREOF

(57) Abstract: The subject invention relates to the identification of four genes involved in the elongation of polyunsaturated acids (i.e., "elongases") and to uses thereof. Two of these genes are also involved in the elongation of monounsaturated fatty acids. In particular, elongase is utilized in the conversion of gamma linolenic acid (GLA) to dihomogama linolenic acid (DGLA) and in the conversion of DGLA or 20:4n-3 to eicosapentaenoic acid (EPA). DGLA may be utilized in the production of polyunsaturated fatty acids, such as arachidonic acid (AA), docosahexaenoic acid (DHA), EPA, adrenic acid, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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ELONGASE GENES AND USES THEREOF

BACKGROUND OF THE INVENTION

Technical Field

The subject invention relates to the identification of several genes involved in the elongation of long-chain polyunsaturated fatty acids (i.e., "elongases") and to uses thereof. In particular, the elongase enzyme is utilized in the conversion of one fatty acid to another. For example, elongase catalyzes the conversion of gamma linolenic acid (GLA) to dihomo-γ-linolenic acid (DGLA, 20:3n-6) and the conversion of stearidonic acid (STA, 18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Elongase also catalyzes the conversion of arachidonic acid (AA, 20:4n-6) to adrenic acid (ADA, 22:4n-6), the conversion of eicosapentaenoic acid (EPA, 20:5n-3) to ω3-docosapentaenoic acid (22:5n-3), and the conversation of α-linolenic acid (ALA, 18:3n-3) to 20:3n-3. DGLA, for example, may be utilized in the production of other polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

Background Information

The elongases which have been identified in the past differ in terms of the substrates upon which they act. Furthermore, they are present in both animals and plants. Those found in mammals have the ability to act on saturated, monounsaturated and polyunsaturated fatty acids. In contrast, those found in plants are specific for saturated or monounsaturated fatty acids. Thus, in order to generate polyunsaturated fatty acids in plants, there is a need for a PUFA-specific elongase.

In both plants and animals, the elongation process is believed to be the result of a four-step mechanism (Lassner et al., The Plant Cell 8:281-292 (1996)). CoA is the acyl carrier. Step one involves condensation of malonyl-CoA with a long-chain acyl-CoA to yield carbon dioxide and a β-ketoacyl-CoA in which the acyl moiety has been elongated by two carbon atoms. Subsequent reactions include reduction to β-hydroxyacyl-CoA, dehydration to an enoyl-CoA, and a second reduction to yield the elongated acyl-CoA. The initial condensation reaction is not only the substrate-specific step but also the rate-limiting step.

As noted previously, elongases, more specifically, those which utilize PUFAs as substrates, are critical in the production of long-chain polyunsaturated fatty acids which have many important functions. For example, PUFAs are important components of the plasma membrane of a cell where they are found in the form of phospholipids. They also serve as precursors to mammalian prostacyclins, eicosanoids, leukotrienes and prostaglandins. Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, efficiently.

A number of enzymes are involved in PUFA biosynthesis including elongases (elo) (see Figure 1). For example, linoleic acid (LA, 18:2-Δ9,12 or 18:2n-6) is produced from oleic acid (OA, 18:1-Δ9 or 18:1n-9) by a Δ12 desaturase. GLA (18:3-Δ6,9,12) is produced from linoleic acid by a Δ6-desaturase. AA (20:4-Δ5,8,11,14) is produced from dihomo-γ-linolenic acid (DGLA, 20:3-Δ8,11,14) by a Δ5-desaturase. As noted above, DGLA is produced from GLA by an elongase.

It must be noted that animals cannot desaturate beyond the Δ9 position and therefore cannot convert oleic acid into linoleic acid. Likewise, α-linolenic acid (ALA, 18:3-Δ9,12,15 or 18:3n-3) cannot be synthesized by mammals, since they lack Δ15 desaturase activity. However, α-linolenic acid can be converted to stearidonic acid (STA, 18:4-Δ6,9,12,15) by a Δ6-desaturase (see PCT publication WO 96/13591; see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4-Δ8,11,14,17 or 20:4n-3) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4-Δ8,11,14,17) can then be converted to eicosapentaenoic acid (EPA, 20:5-Δ5,8,11,14,17) by a Δ5-desaturase. Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbons 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,974) and 15 (see PCT publication WO 93/11245). The major polyunsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or α-linolenic acid. In view of the inability of mammals to produce these essential long chain fatty acids, it is of significant interest to isolate genes involved in PUFA biosynthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant or animal system which can be altered to provide production of commercial quantities of one or more PUFAs. Consequently, there is a definite need for the elongase enzyme, the gene encoding the enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFA beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the elongase gene.

One of the most important long chain PUFAs, noted above, is arachidonic acid (AA). AA is found in filamentous fungi and can also be purified from mammalian tissues including the liver and the adrenal glands. As noted above, AA production from DGLA is catalyzed by a Δ5-desaturase, and DGLA production from γ-linolenic acid (GLA) is catalyzed by an elongase. However, until the present invention, no elongase had been identified which was active on substrate fatty acids in the pathways for the production of long chain PUFAs and, in particular, AA, eicosapentaenoic acid (EPA), adrenic acid, docosahexaenoic acid (DHA, 22:6n-3), ω3-docosapentaenoic acid (22:5n-3) or ω6-docosapentaenoic acid (22:5n-6).

Two genes appeared to be of interest in the present search for the elongase gene. In particular, the jojoba β-ketoacyl-coenzyme A synthase (KCS), or jojoba KCS (GenBank Accession # U37088), catalyzes the initial reaction of the fatty acyl-CoA elongation pathway (i.e., the condensation of malonyl-CoA with long-chain acyl-CoA (Lassner et al., The Plant Cell 8:281-292 (1996)). Jojoba KCS substrate preference is 18:0, 20:0, 20:1, 18:1, 22:1, 22:0 and 16:0. Saccharomcyes cerevisiae elongase (ELO2) also catalyzes the conversion of long chain saturated and monounsaturated fatty acids, producing high levels of 22:0, 24:0, and also 18:0, 18:1, 20:0, 20:1, 22:0, 22:1, and 24:1 (Oh et al., The Journal of Biological Chemistry 272 (28):17376-17384 (1997); see also U.S. Patent No. 5,484,724 for a nucleotide sequence which includes the sequence of ELO2; see PCT publication WO 88/07577 for a discussion of the sequence of a glycosylation inhibiting factor which is described in Example V). The search for a long chain PUFA-specific elongase in Mortierella alpina began based upon a review of the homologies shared between these two genes and by expression screening for PUFA-elongase activity.

Summary of the Invention

The present invention relates to an isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence shown in SEQ ID NO:1 (Figure 6), wherein said isolated nucleotide sequence encodes a polypeptide having elongase activity. This isolated sequence may be represented by SEQ ID NO:1. The sequence encodes a functionally active elongase which utilizes a polyunsatured fatty acid or a monounsaturated fatty acid as a substrate. In particular, the sequence may be derived from a fungus of the genus Mortierella and may specifically be isolated from Mortierella alpina.

The present invention also includes a purified protein encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsatured fatty acids or monounsaturated fatty acids and has at least about 50% amino acid similarity to the amino acid sequence of the purified protein encoded by the above nucleotide sequence.

Additionally, the present invention encompasses a method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO: 1 (Figure 6); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The host cell may be a eukaryotic cell or a prokaryotic cell.

The prokaryotic cell may be, for example an E. coli cell, a cyanobacterial cell, or a B. subtilis cell. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell. The fungal cell may be, for example, Saccharomyces sp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula

spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. or Pichia spp. In particular, the fungal cell may be a yeast cell such as Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. or Pichia spp.

The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 6) operably linked to b) a promoter, as well as a host cell comprising this vector. The host may be a prokaryotic cell or a eukaryotic cell. Suitable examples of prokaryotic cells include E. coli, Cyanobacteria, and B. subtilis cells. Suitable examples of eukaryotic cells include a mammalian cell, an insect cell, a plant cell and a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp. In particular, the fungal cell may be, for example, a yeast cell such as, for example, Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. and Pichia spp.

The present invention includes a plant cell, plant or plant tissue comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, dihomo-γ-linolenic acid (DGLA), 20:4n-3, and adrenic acid (ADA). The invention also includes one or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue. Additionally, the present invention encompasses a transgenic plant comprising the above-described vector,

wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Furthermore, the present invention includes a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:1 (Figure 6). The present invention also includes a fluid (e.g., milk) produced by the transgenic, non-human wherein the fluid comprises a detectable level of at least one elongase or products thereof such as, for example, DGLA, ω6-docosapentaenoic acid, ADA and/or 20:4n-3 (see Figure 1).

Additionally, the present invention includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 6); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a "substrate" polyunsaturated fatty acid in order to convert the substrate to a "product" polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be selected from the group consisting of, for example, γ-linolenic acid (GLA), stearidonic acid (STA) and arachidonic acid (AA), and the product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to "another" polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group

consisting of, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, eicosapentaenoic acid (EPA), ω6-docosapentaenoic acid, respectively, and the at least one desaturase is Δ5-desaturase, with respect to production of AA or EPA, and Δ4-desaturase, with respect to production of ω6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a "final" polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid (DHA), AA, ω6-docosapentaenoic acid, or ω3-docosapentaenoic acid.

Also, the present invention includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid. The nutritional composition may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or an animal and may be administered enterally or

parenterally. The nutritional composition may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil. It may also comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

Additionally, the present invention encompasses a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method of claim 32, and the final polyunsaturated fatty acid produced according to the above-described method and 2) a pharmaceutically acceptable carrier. The composition may be administered to a human or an animal. It may also further comprise at least one element selected from the group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound. It may be administered enterally, parenterally, topically, rectally, intramuscularly, subcutaneously, intradermally, or by any other appropriate means.

The present invention also includes an animal feed

comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid.

Moreover, the present invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method and the final polyunsaturated fatty acid produced according to the above-described method.

Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the above nutritional composition in an amount sufficient to effect prevention or treatment.

The present invention also includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO: 2 (Figure 22) wherein said isolated nucleotide sequence encodes a polypeptide having enlongase activity. This sequence may be represented by SEQ ID NO: 2. The sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. This sequence may also be derived, for example, from a fungus

of the genus Mortierella. In particular, it may be derived from M. alpina.

Additionally, the present invention includes a purified protein encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

The present invention also includes a method of producing an elongase enzyme as described above. The sequence inserted in the vector is represented by SEQ ID NO:2 (Figure 22). The host cell may be prokaryotic or eukaryotic. Suitable examples are described above.

The present invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:2 (Figure 22) operably linked to b) a promoter, as well as a host cell comprising this vector. Again, the host cell may be eukaryotic or prokaryotic. Suitable examples are described above.

The invention also includes a plant cell, plant or plant tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. Additionally, the invention includes one or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue.

Furthermore, the present invention also includes a transgenic plant comprising the above vector, wherein expression of the nucleotide sequence (SEQ ID NO:2) of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

The invention also includes a transgenic, non-human mammal whose genome comprises a DNA sequence (SEQ ID NO:2) encoding an elongase operably linked to a promoter. The invention also includes a fluid produced by this transgenic, non-human mammal wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The present invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:2 (Figure 22); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA, or AA, the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ω6-docosapentaenoic acid, respectively. The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA, or ω6-docosapentaenoic acid, respectively, and the at least one desaturase is Δ5-desaturase with respect to production of AA or EPA, and Δ4-desaturase with respect to production of ω6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in

order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid, AA, ω6-docosapentaenoic acid, or ω3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, EPA, and ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be selected from the group consisting of, for example, DHA, AA, ω6-docosapentaenoic acid, and ω3-docosapentaenoic acid. The other attributes of the composition are the same as those described above with respect to administration, characterization, components, etc.

The present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method of noted above with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and 2) a pharmaceutically acceptable carrier. The characteristics of the above-described pharmaceutical composition (e.g.,

administration, components, etc.) also apply to this composition.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid.

The invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2.

Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition described directly above in an amount sufficient to effect the prevention or treatment.

Furthermore, the present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID

NO:3 (Figure 43) wherein said isolated nucleotide sequence encodes a polypeptide having elongase activity. This sequence may be that represented by SEQ ID NO:3. This sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. The sequence is derived from a mammal such as, for example, a human.

The invention also includes a purified protein encoded by this nucleotide sequence. Also, the invention includes a purified polypeptide which elongates polyunsaturated fatty ackds or monounsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of this purified protein.

Additionally, the invention includes method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing said vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The host cell may be the same as that described above with respect to the corresponding methods utilizing SEQ ID NO:1 or 2.

The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:3 (Figure 43) operably linked to b) a promoter, as well as a host cell comprising this vector. The host cell may be the same as that described above.

The invention also includes a plant cell, plant or plant tissue comprising the above-described vector comprising SEQ ID NO:3, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by said plant cell, plant or plant tissue. The polyunsaturated fatty acid may

be, for example, DGLA, 20:4n-3 or ADA. The invention also includes one or more plant oils or acids expressed by the plant cell, plant or plant tissue.

The invention also includes a transgenic plant comprising the vector comprising SEQ ID NO:3, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a human DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence is represented by SEQ ID NO:3 (Figure 43). The invention also includes a fluid produced by said transgenic, non-human mammal wherein said fluid comprises a detectable level of at least one elongase or products thereof.

The invention also encompasses a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b) constructing a vector comprising said nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA or AA, and the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product

polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 and ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA, and ω6-docosapentaenoic acid, respectively, and the at least one desaturase is Δ5-desaturase with respect to production of AA or EPA and Δ4-desaturase with respect to production of ω6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA, ω6-docosapentaenoic acid, and ω3-docosapentaenoic acid.

The nutritional composition comprising at least one polyunsaturated fatty acid which may be, for example, product polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be selected from the group consisting of AA, EPA, or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA, ω6-docosapentaenoic acid, or ω3-docosapentaenoic acid. The other properties or characteristic of the nutritional composition (e.g., administration, components, etc.) as the same as those recited above with respect to the other nutritional compositions.

Moreover, the present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3, the another polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3 and 2) a pharmaceutically acceptable carrier. The other properties of the composition (e.g., administration, additional components, etc.) are the same as those recited above with respect to the other pharmaceutical compositions.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, the another polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The polyunsaturated fatty acid may be, for example, AA, EPA, or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid.

Also, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, said another polyunsaturated fatty acid produced according to the method recited above in connection

with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited in connection with SEQ ID NO:3.

A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional composition recited above in connection with SEQ ID NO: 3 in an amount sufficient to effect the prevention or treatment.

Additionally, the present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO: 4 (Figure 46) wherein said isolated nucleotide sequence encodes a polypeptide having elongase activity. The sequence may be represented by SEQ ID NO: 4. It encodes a functionally active elongase which utilizes a polyunsatured fatty acid as a substrate. The sequence may be derived or isolated from a nematode of the genus Caenorhabditis and, in particular, may be isolated from C. elegans.

The present invention includes a purified protein encoded by the nucleotide sequence above. The invention also includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

Additionally, the present invention includes a method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO: 4 (Figure 46); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The properties of the host cell are the same as those described above in connection with SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

The present include also encompasses a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:4 (Figure 46) operably linked to b) a promoter, as well as a host cell comprising this vector. The host cell has the same properties as those recited above in connection with the host cell recited above for SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

Moreover, the present invention includes a plant cell, plant or plant tissue comprising the above vector comprising SEQ ID NO:4, wherein expression of said nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The invention also includes one or more plant oils or fatty acids expressed by this plant cell, plant or plant tissue.

The invention also includes transgenic plant comprising the above vector including the nucleotide sequence corresponding to SEQ ID NO:4, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a C. elegans DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:4 (Figure 46). The invention also includes a fluid produced by the transgenic, non-human mammal of claim 187 wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:4 (Figure 46); constructing a vector comprising the isolated

nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA, or AA, and the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, respectively. The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA or ω6-docosapentaenoic acid, respectively, and the at least one desaturase is Δ5-desaturase with respect to production of AA or EPA, and Δ4-desaturase with respect to production of ω6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA, ω6-docosapentaenoic acid, or ω3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said the polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method described above

in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA, ω6-docosapentaenoic acid, or ω3-docosapentaenoic acid. The other characteristics of the composition are the same as those recited for the nutritional compositions present above.

Additionally, the present invention includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4 and 2) a pharmaceutically acceptable carrier. The composition has the same properties (e.g., administration, added elements, etc.) as those described above with respect to the other pharmaceutical compositions.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The

another polyunsaturated fatty acid may be, for example, AA, EPA or ω6-docosapentaenoic acid. The polyunsaturated fatty acid may be, for example, DHA, ADA, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid.

Additionally, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4 and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4.

Furthermore, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition recited with respect to SEQ ID NO:4 in an amount sufficient to effect the treatment or prevention.

All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents various fatty acid biosynthesis pathways. The role of the elongase enzyme (elo) should be noted.

Figure 2 represents the percent similarity and percent identity between the amino acid sequences of jojoba KCS and ELO2. (Gap Weight: 6; Average Match: 2.912;
Length Weight: 4; Average Mismatch: -2.003;
Quality: 50; Length: 84;
Ratio: 0.625; Gaps: 4;
Percent Similarity: 43.038; Percent Identity: 29.114)

(Match display thresholds for the alignment(s):
| = IDENTITY; : = 2; . = 1)

Figure 3 represents the S. cerevisiae ELO2 sequence homologous to the jojoba KCS sequence (primer sequence underlined) of Figure 2.

S. cerevisiae ELO2 (AA66-145) with M. Alpina codon bias

Figure 4A shows the physical map of pRAE-2 containing the MAELO cDNA. Figure 4B represents the physical map of the constitutive expression vector, pRAE-5, used for elongase enzyme production in yeast.

Figure 5 represents a comparison of the nucleotide sequences of clones pRAE-5 and pRAE-6.

Figure 6 illustrates the complete nucleotide sequence of Mortierella alpina elongase (MAELO).

Figure 7 represents the amino acid sequence of the Mortierella alpina elongase translated from MAELO (see Figure 6).

Figure 8 represents an amino acid sequence alignment among 3 elongases: S. cerevisiae ELO2 (GNS1), S. cerevisiae ELO3 (SUR4) and the translated MAELO sequence as shown in Figure 7.

Figure 9 represents a comparison between the nucleotide sequence MAELO and the nucleotide sequence of ELO2 from S. cerevisiae. SCORES Initl: 153 Initn: 199 Opt: 495
57.4% identity in 549 bp overlap

Figures 10A and 10B represents the PUFA elongase activity of MAELO expressed in baker's yeast.

Figure 11 illustrates the PUFA elongase activity of MAELO when co-expressed with the Δ5-desaturase cDNA from M. alpina to produce AA.

Figure 12 compares the PUFA elongase activity of MAELO to the overexpression of ELO2 from S. cerevisiae in baker's yeast.

Figures 13, 14 and 15 represent three separate comparisons of amino acid sequences derived from C. elegans nucleotide sequences in the GenEMBL database with the translated MAELO.

SCORES Initl: 156 Initn: 215 Opt: 296
Smith-Waterman score: 296; 28.8% identity in 264 aa overlap

SCORES Initl: 178 Initn: 178 Opt: 318
Smith-Waterman score: 318; 33.0% identity in 188 aa overlap

SCORES Initl: 30 Initn: 30 Opt: 40
Smith-Waterman score: 49; 22.1% identity in 86 aa overlap

Figure 16 shows the comparison between amino acid translations of two different mammalian sequences in the GenEMBL database and the translated MAELO.

MOUSE
SCORES Initl: 161 Initn: 191 Opt: 325
Smith-Waterman score: 325; 28.8% identity in 285 aa overlap

HUMAN
SCORES Initl: 147 Initn: 147 Opt: 211
Smith-Waterman score: 211; 28.7% identity in 150 aa overlap

Figure 17 shows the comparison of a translated DNA sequence (see published PCT application WO 88/07577) with the amino acid sequence derived from MAELO, which was detected during a database search.

SCORES Initl: 87 Initn: 218 Opt: 232
Smith-Waterman score: 272; 29.7% identity in 232 aa overlap

Figure 18 shows the complete nucleotide sequence of the Δ5-desaturase from M. alpina.

Figure 19 represents the initial GC-FAME analysis of MAD708 pool. The detection of a DGLA (C20:3n-6) peak should be noted.

Figure 20 represents the PUFA elongase activity of the five MAD708 clones in yeast with GLA as substrate. All clones have apparent elongase activity.

Figure 21 represents the DNA sequencing analysis of plasmid pRPB2. The analysis reveals an open reading frame of 957 bp in length.

Figure 22 shows the complete nucleotide sequence of the M. alpina cDNA, contained in the plasmid pRPB2, which is designated GLELO for its GLA elongase activity.

Figure 23 represents the amino acid sequence of the M. alpina elongase translated from GLELO (see Figure 22).

Figure 24 illustrates the n-6 PUFA elongase activity in an induced culture of 334(pRPB2) when supplemented with GLA.

Figure 25 represents the n-3 and n-6 PUFA elongase activity in an induced culture of 334(pRPB2) when supplemented with 25 μm of other fatty acid substrates.

Figure 26A illustrates the elongase activity of GLELO with GLA as a substrate when co-expressed with the M. alpina Δ5-desaturase cDNA to produce AA. Figure 26B illustrates the elongase activity of GLELO with STA as a substrate when co-expressed with the M. alpina Δ5-desaturase cDNA to produce EPA.

Figure 27 illustrates the comparison between the translated GLELO sequence (see Figure 23) and the translated MAELO sequence (see Figure 7).

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SCORES      Initl: 114   Initn: 278   Opt:  278
Smith-Waterman score: 308;    30.9% identity in 259 aa overlap
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Figure 28 represents a comparison of the amino acid sequence of 4 elongases: the translated amino acid sequence of GLELO (see Figure 23), MAELO (see Figure 7), S. cerevisiae ELO2 (GNS1), and S. cerevisiae ELO3 (SUR4). The histidine box is underlined.

Figure 29 represents an alignment between translated MAELO sequence and translated putative human homologue HS1 sequence.

SCORES Initl: 83 Initn: 186 Opt: 271
Smith-Waterman score: 297; 28.5% identity in 242 aa overlap

Figure 30 represents an alignment between the translated MAELO sequence and the translated putative human homologue HS2 sequence.

SCORES Initl: 88 Initn: 208 Opt: 272
Smith-Waterman score: 279; 28.2% identity in 266 aa overlap

Figure 31 shows an alignment between the translated MAELO sequence and the translated putative mouse homologue MM2 sequence.

SCORES Initl: 88 Initn: 207 Opt: 223
Smith-Waterman score: 236; 30.4% identity in 191 aa overlap

Figure 32 represents an alignment between the translated MAELO and the translated putative mouse homologue AI225632 sequence.

SCORES Initl: 51 Initn: 115 Opt: 168
Smith-Waterman score: 168; 30.4% identity in 115 aa overlap

Figure 33 illustrates an alignment between the translated GLELO sequence and the translated human homologue AI815960 sequence.

SCORES Frames: (3) Initl: 332 Initn: 332 Opt: 384
40.3% identity in 144 aa overlap

Figure 34 shows an alignment between the translated GLELO sequence and the translated putative human homologue HS1 sequence.

SCORES Initl: 316 Initn: 384 Opt: 477
Smith-Waterman score: 477; 34.2% identity in 240 aa overlap

Figure 35 represents an alignment between the translated GLELO sequence and the translated putative human homologue sequence from AC004050.

SCORES Initl: 80 Initn: 114 Opt: 178
Smith-Waterman score: 178; 28.8% identity in 146 aa overlap

Figure 36 illustrates an alignment between the translated GLELO sequence and the translated putative mouse homologue MM2 sequence.

SCORES Initl: 288 Initn: 288 Opt: 399
Smith-Waterman score: 399; 34.6% identity in 211 aa overlap

Figure 37 represents an alignment of the translated GLELO sequence and a translated putative mouse homologue AI225632 sequence.

SCORES Initl: 160 Initn: 227 Opt: 269
Smith-Waterman score: 269; 35.3% identity in 119 aa overlap

Figure 38 illustrates an alignment of the translated GLELO sequence and a translated putative mouse homologue U97107.

SCORES Initl: 64 Initn: 129 Opt: 233
Smith-Waterman score: 239; 23.7% identity in 279 aa overlap

Figure 39 represents an alignment of the translated GLELO sequence and a translated putative C. elegans U68749 (F56H11.4) homologue sequence.

SCORES Initl: 100 Initn: 205 Opt: 271
Smith-Waterman score: 271; 30.7% identity in 218 aa overlap

Figure 40 shows an alignment between the translated MAELO sequence and a translated putative C. elegans U68749 (F56H11.4) homologue sequence.

SCORES Initl: 189 Initn: 264 Opt: 358
Smith-Waterman score: 358; 28.7% identity in 296 aa overlap

Figure 41 represents an alignment between the translated GLELO sequence and a translated putative Drosophila melanogaster homologue sequence, DM1.

SCORES Initl: 77 Initn: 155 Opt: 264
Smith-Waterman score: 264; 27.2% identity in 206 aa overlap

Figure 42 illustrates an alignment between the translated MAELO sequence and a translated putative Drosophila melanogaster homologue sequence, DM1.

SCORES Initl: 181 Initn: 279 Opt: 328
Smith-Waterman score: 328; 30.0% identity in 237 aa overlap

Figure 43 illustrates the complete nucleotide sequence of a human elongase HSELO1.

Figure 44 represents the deduced amino acid sequence of the human elongase HSELO1.

Figure 45 illustrates the elongase activity (PUFA and others) of an induced culture of 334(pRAE-58-A1) when supplemented with GLA or AA.

Figure 46 shows the complete nucleotide sequence of the C. elegans elongase CEELO.

Figure 47 represents the deduced amino acid of C. elegans elongase CEELO.

Figure 48 illustrates the PUFA elongase activity of an induced culture of 334(pRET-21) and 334(pRET-22) when supplemented with GLA and AA.

Figure 49 represents the complete nucleotide sequence of the putative human elongase gene HS3.

Figure 50 illustrates the deduced amino acid sequence of the putative human elongase enzyme HS3.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to nucleotide and corresponding amino acid sequences of two elongase cDNAs

derived from Mortierella alpina, as well as to nucleotide and corresponding amino acid sequences of an elongase cDNA derived from a human and one derived from C. elegans. Furthermore, the subject invention also includes uses of the cDNAs and of the proteins encoded by the genes. For example, the genes and corresponding enzymes may be used in the production of polyunsaturated fatty acids and/or monounsaturated fatty acids such as DGLA, AA, ADA, EPA and/or DHA which may be added to pharmaceutical compositions, nutritional compositions and to other valuable products.

The Elongase Genes and Enzymes Encoded Thereby

As noted above, an elongase enzyme encoded by an elongase cDNA is essential in the production of various polyunsaturated fatty acids, in particular, 20-24 carbon PUFAs. With respect to the present invention, the nucleotide sequence of the isolated M. alpina elongase cDNA (MAELO) is shown in Figure 6, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this nucleotide sequence is shown in Figure 7. Additionally, the nucleotide sequence of the isolated GLA elongase cDNA (GLELO) is shown in Figure 22, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this nucleotide sequence is shown in Figure 23. The nucleotide sequence of the isolated human sequence 1 (HSELO1) elongase is shown in Figure 43, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this sequence is shown in Figure 44. Furthermore, the nucleotide sequence of the isolated C. elegans elongase cDNA (CEELO1) is shown in Figure 46, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 47.

As an example, the isolated elongases encoded by the cDNAs of the present invention elongate GLA to DGLA or elongate STA to 20:4n-3 or elongate AA to ADA. The production of arachidonic acid from DGLA, or EPA from 20:4n-3, is then catalyzed by a Δ5-desaturase. Thus, neither AA (or EPA), nor DGLA (or 20:4n-3) nor ADA (or ω3-docosapentaenoic acid), can be synthesized without at least one elongase cDNA and enzyme encoded thereby.

It should be noted that the present invention also encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 50%, preferably at least about 60%, and more preferably at least about 70% of the nucleotides in SEQ ID NO:1 (i.e., the nucleotide sequence of the MAELO cDNA described herein (see Figure 6)). Furthermore, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:2 (i.e., the nucleotide sequence of the GLELO cDNA described herein (see Figure 22). Additionally, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:3 (i.e., the nucleotide sequence of the human sequence 1 (HSELO1) cDNA described herein (see Figure 43). In addition, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least

about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:4 (i.e., the nucleotide sequence of the C. elegans cDNA, CEELO1, described herein (see Figure 46). Such sequences may be derived from non-Mortierella sources (e.g., a eukaryote (e.g., Thraustochytrium spp. (e.g., Thraustochytrium aureum and Thraustochytrium roseum), Schizochytrium spp. (e.g., Schizochytrium aggregatum), Conidiobolus spp. (e.g., Conidiobolus nanodes), Entomorphthora spp. (e.g., Entomorphthora exitalis), Saprolegnia spp. (e.g., Saprolegnia parasitica and Saprolegnia diclina), Leptomitus spp. (e.g., Leptomitus lacteus), Entomophthora spp., Pythium spp., Porphyridium spp. (e.g., Porphyridium cruentum), Conidiobolus spp., Phytophathora spp., Penicillium spp., Coidosporium spp., Mucor spp. (e.g., Mucor circinelloides and Mucor javanicus), Fusarium spp., Aspergillus spp. and Rhodotorula spp.), a yeast (e.g., Dipodascopsis uninucleata), a non-mammalian organism such as a fly (e.g., Drosophila melanogaster) or Caenorhabditis spp. (e.g., Caenorhabditis elegans), or a mammal (e.g., a human or a mouse). Such sequences may be derived from species within the genus Mortierella, other than the species alpina, for example, Mortierella elongata, Mortierella exigua, Mortierella isabellina, Mortierella hygrophila, and Mortierella ramanniana, va. angulispora. Furthermore, the present invention also encompasses fragments and derivatives of the nucleotide sequences of the present invention (i.e., SEQ ID NO:1 (MAELO), SEQ ID NO:2 (GLELO), SEQ ID NO:3 (HSELO1) and SEQ ID NO:4 (CEELO1)), as well as of the sequences derived from non-Mortierella sources and having the above-described complementarity or correspondence/identity. Functional equivalents of the above-sequences (i.e., sequences having elongase activity) are also encompassed by the present invention.

For purposes of the present invention, "complementarity" is defined as the degree of relatedness between two DNA segments. It is determined by measuring the ability of the sense strand of one DNA segment to hybridize with the antisense strand of the other DNA segment, under appropriate conditions, to form a double helix. In the double helix, wherever adenine appears in one strand, thymine appears in the other strand. Similarly, wherever guanine is found in one strand, cytosine is found in the other. The greater the relatedness between the nucleotide sequences of two DNA segments, the greater the ability to form hybrid duplexes between the strands of two DNA segments.

"Identity" between two nucleotide sequences is defined as the degree of sameness, correspondence or equivalence between the same strands (either sense or antisense) of two DNA segments. The greater the percent identity, the higher the correspondence, sameness or equivalence between the strands.

"Similarity" between two amino acid sequences is defined as the presence of a series of identical as well as conserved amino acid residues in both sequences. The higher the degree of similarity between two amino acid sequences, the higher the correspondence, sameness or equivalence of the two sequences. ("Identity" between two amino acid sequences is defined as the presence of a series of exactly alike or invariant amino acid residues in both sequences.)

The definitions of "complementarity", "identity", and "similarity" are well known to those of ordinary skill in the art.

The invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and has at least about 50% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure

7 (MAELO)) and which are, in turn, encoded by the above-described nucleotide sequences. Additionally, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 23 (GLELO)) and which are, in turn, encoded by the above-described nucleotide sequences. Furthermore, the invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 44 (HSELO1)) and which are, in turn, encoded by the above-described nucleotide sequences. Also, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 47 (CEELO1)) and which are, in turn, encoded by the above-described nucleotide sequences.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA elongase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence corresponding or complementary to the nucleotide sequence represented by SEQ ID NO:1 shown in Figure 6 (MAELO) and/or SEQ ID NO:2 shown in Figure 22 (GLELO) and/or SEQ ID NO:3 (HSELO1) shown in Figure 43 and/or SEQ ID NO:4 (CEELO1) shown in Figure 46. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory

Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementarity. Such variables are well known in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of Tm, melting temperature, for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook et al., supra). For hybridization with shorter nucleic acids, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., supra).

Production of the Elongase Enzyme

Once the gene encoding the elongase has been isolated, it may then be introduced into either a prokaryotic or eukaryotic host cell through the use of a vector, plasmid or construct.

The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the elongase as well as any promoter which is functional in the host cell and is able to elicit expression of the elongase encoded by the nucleotide sequence. The promoter is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects transcription or expression of the coding sequence.)

Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucoisomerase, phosphoglycerate kinase, acid phosphatase, T7, TP1, lactase, metallothionein, cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T-antigen, ovalalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired expression products as well as the nature of the host cell.

As noted above, once the vector has been constructed, it may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for example, transfection, transformation and electroporation (see Molecular Cloning: A Laboratory Manual, 2nd ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)). The host cell is then cultured under suitable conditions permitting expression of the PUFA which is then recovered and purified.

It should also be noted that one may design a unique triglyceride or oil if one utilizes one construct or vector comprising the nucleotide sequences of two or more cDNAs (e.g., MAELO, GLELO, HSELO1 and/or CEELO1). This vector may then be introduced into one host cell. Alternatively, each of the sequences may be introduced into a separate vector. These vectors may then be introduced into two host cells, respectively, or into one host cell.

Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells include, for example, mammalian cells, plant cells, yeast cells such as Saccharomyces spp., Lipomyces spp., Candida spp. such as Yarrowia (Candida) spp., Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such as filamentous fungal cells, for example, Aspergillus, Neurospora and Penicillium. Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, the site of the construct's integration can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or

some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

A transgenic mammal may also be used in order to express the enzyme of interest (i.e., the elongase) encoded by one or both of the above-described nucleotide sequences. More specifically, once the above-described construct is created, it may be inserted into the pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al., Science 278:2130-2133 (1997)). Gestation and birth are then permitted to occur(see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFAs, as compared to the levels normally found in the non-transgenic animal. Subsequent generations may be monitored for production of the altered or enhanced levels of PUFAs and thus incorporation of the gene or genes encoding the elongase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse, a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the ability to incorporate DNA encoding the enzyme of interest into its genome.

For expression of an elongase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the elongase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a

plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific regulatory sequence such as those of U.S. Patent Nos. 5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,589,379, Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of an elongase gene or genes, or antisense elongase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The elongase polypeptide coding region may be expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected as a matter of convenience rather than because of any particular property.

As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)), plant tissue, corn, potatoe, sunflower, safflower or flax may also be utilized as a host or host cell, respectively, for expression of the elongase enzyme(s) which may, in turn, be utilized in the production of polyunsaturated fatty acids. More specifically, desired PUFAs can be expressed in seed. Methods of isolating seed

oils are known in the art. Thus, in addition to providing a source for PUFAs, seed oil components may be manipulated through the expression of the elongase genes, as well as perhaps desaturase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA sequence encoding the elongase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the elongase gene. The vector may also comprise one or more genes which encode other enzymes, for example, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ10-desaturase, Δ12-desaturase, Δ13-desaturase, Δ15-desaturase, Δ17-desaturase and/or Δ19-desaturase. The plant tissue or plant may produce the relevant substrate (e.g., DGLA, GLA, STA, AA, ADA, EPA, 20:4n-3, etc.) upon which the enzymes act or a vector encoding enzymes which produce such substrates may be introduced into the plant tissue, plant cell, plant, or host cell of interest. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFAs (e.g., n-6 unsaturated fatty acids such as DGLA, AA or ADA, or n-3 fatty acids such as EPA or DHA) by use of a plant cell, plant tissue, plant, or host cell of interest. It should also be noted that the invention also encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector,

which is subsequently introduced into the host cell, are shown in Figure 1.

In view of the above, the present invention also encompasses a method of producing one of the elongase enzymes described above comprising the steps of: 1) isolating the desired nucleotide sequence of the elongase cDNA; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said vector into a host cell under time and conditions sufficient for the production of the elongase enzyme.

The present invention also encompasses a method of producing polyunsaturated fatty acids comprising exposing an acid to the elongase(s) produced as above such that the elongase converts the acid to a polyunsaturated fatty acid. For example, when GLA is exposed to elongase, it is converted to DGLA. DGLA may then be exposed to Δ5-desaturase which converts the DGLA to AA. The AA may then be converted to EPA by use of Δ17-desaturase which may be, in turn, converted to DHA by use of elongase and a Δ4-desaturase. Alternatively, elongase may be utilized to convert 18:4n-3 to 20:4n-3 which may be exposed to Δ5-desaturase and converted to EPA. Elongase may also be used to convert 18:3n-3 to 20:3n-3, which may be, in turn, converted to 20:4n-3 by a Δ8-desaturase. Thus, elongase may be used in the production of polyunsaturated fatty acids which may be used, in turn, for particular beneficial purposes. (See Figure 1 for an illustration of the many critical roles elongase plays in several biosynthetic pathways.)

Uses of the Elongase Gene and Enzyme Encoded Thereby

As noted above, the isolated elongase cDNAs and the corresponding elongase enzymes (or purified polypeptides) encoded thereby have many uses. For example, each cDNA and

corresponding enzyme may be used indirectly or directly in the production of polyunsaturated fatty acids, for example, DGLA, AA, ADA, 20:4n-3 or EPA. ("Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter of which is utilized in a composition (e.g., the conversion of GLA to DGLA)). "Indirectly" is meant to encompass the situation where a fatty acid is converted to another fatty acid (i.e., a pathway intermediate) by elongase (e.g., GLA to DGLA) and then the latter fatty acid is converted to another fatty acid by use of a non-elongase enzyme (e.g., DGLA to AA by Δ5-desaturase)). These polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the elongase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses are described, in detail, below.

Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least one oil or acid produced by use of at least one elongase enzyme, produced using the respective elongase gene, and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for

a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants,

specialist infant formulae, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

The nutritional composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutrional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott

Laboratories, Columbus, Ohio). An oil or fatty acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form, the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and, more preferably, less than 660 mOsm.

The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the PUFAs produced in accordance with the present invention. The presence of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to the provision of PUFAs, it may also be desirable to add zinc, copper, folic acid and antioxidants to the composition. It is believed that these substance boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may also be supplemented with these elements.

In a more preferred embodiment, the nutritional composition comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight % of the carbohydrate is indigestible oligosaccharide. In a more preferred embodiment, the nutritional composition additionally comprises protein, taurine, and carnitine.

As noted above, the PUFAs produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant formula, for patients undergoing intravenous feeding or for

preventing or treating malnutrition or other conditions or disease states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% as EPA, from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus, fatty acids such as DGLA, AA, EPA and/or docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of AA, DGLA and GLA. More preferably, the oil blend will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For

example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to AA can be used to produce an AA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% to 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of elongase expression, as well as the expression of other desaturases, can be used to modulate PUFA levels and ratios. The PUFAs/acids produced in accordance with the present invention (e.g., AA and DGLA) may then be combined with other PUFAs/acids (e.g., GLA) in the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the fatty acids and/or resulting oils produced using at least one of the elongase cDNAs (i.e., MAELO, GLELO, HSELO1, or CEELO), in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or

vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

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With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

The present invention also includes the treatment of various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the compositions of the present invention may be used to treat restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may also be treated with the compositions of the invention. Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions. Addition of fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may also be useful for treating cachexia associated with cancer.

The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have been demonstrated in diabetic animals.

Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the elongase enzyme(s), may also be used in the treatment of eczema, in the reduction of blood pressure, and in the improvement of mathematics examination scores. Additionally, the compositions of the present

invention may be used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976), reduction or prevention of gastrointestinal bleeding and other side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as well as humans, as animals experience many of the same needs and conditions as humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in animal topical ointments.

The present invention may be illustrated by the use of the following non-limiting examples:

Example I

Determination of Codon Usage in *Mortierella alpina*

The 5' end of 1000 random cDNA clones were sequenced from Mortierella alpina cDNA library. The sequences were translated in six reading frames using GCG (Genetics Computer Group (Madison, Wisconsin)) with the FastA algorithm (Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988)) to search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein), specifically with the Swissprot database (GeneBio, Geneva, Switzerland). Many of the clones were identified as a putative housekeeping gene based on protein sequence homology to known genes. Twenty-one M. alpina cDNA sequences which matched with known, housekeeping genes in the database were selected (see Table 1 below). M. alpina codon bias table (see Table 2) was generated based on these 21 sequences as well as the full length M. alpina Δ5- (see Figure 18), Δ6-, and Δ12-desaturase sequences. Since the FastA alignment between the putative protein coded by the M. alpina cDNA sequence and the known protein sequence was weak in some areas, only the codons from areas of strong homology were used.

Table 1

Clone #	Match	# of bp	# of aa
193	Elongation factor 1-alpha	426	142
143	60S ribosomal protein L17	417	139
235	Actin I	360	120
299	40S ribosomal protein YS11	387	129
390	Ras-related protein rab-1a	342	114
65	40S ribosomal protein RP10	366	122
289	Ubiquitin-conjugating enzyme E2-16 KD	294	98
151	Ubiquinol-cytochrome C reductase	375	125
80	Initiation factor 5A-2	183	61
33	60S ribosomal protein L15	252	84

Table 1 (continued)

Clone #	Match	# of bp	# of aa
132	60S ribosomal protein L3-2	300	100
198	Histone H3	285	95
286	6-phosphogluconate dehydrogenase, decarboxylating	363	121
283	40S ribosomal protein S22	261	87
127	Elongation factor 2	231	77
197	Actin, gamma	252	84
496	40S ribosomal protein S16	270	90
336	Histone H4	219	73
262	Ubiquitin	228	76
188	Guanine nucleotide-binding protein beta subunit-like protein	213	71
81	Ubiquitin	228	76
21	TOTAL	6252	2084

Table 2

Amino acid	Codon Bias	% used	Amino acid	Codon Bias	% used
Ala	GCC	63%	Lys	AAG	96%
Arg	CGC	50%	Met	ATG	100%
Asn	AAC	97%	Phe	TTC	78%
Asp	GAC	65%	Pro	CCC	68%
Cys	TGC	87%	Ser	TCC	46%
Gln	CAG	78%	Thr	ACC	78%
Glu	GAG	85%	Trp	TGG	100%
Gly	GGT	47%	Tyr	TAC	95%
His	CAC	91%	Val	GTC	72%
Ile	ATC	72%	Stop	TAA	50%
Leu	CTC	49%			

Example II

Cloning of a Full-length Elongase-like cDNA from *M. alpina*

The β-ketoacyl-coenzyme A synthase (KCS) from jojoba and the Saccharomyces cerevisiae elongase (ELO2) were aligned to determine an area of amino acid homology (see Figure 2). The codon bias was applied to the area of sequence corresponding to the homologous amino acids between the two elongases, and primers were designed based on this biased sequence (see Figure 3). The cDNA was excised from the M11 M. alpina cDNA library (Knutzon et al., J. Biol. Chem. 273:29360-29366 (1998)), which contains approximately 6 X 10^5 clones with an average insert size of 1.1 Kb. The excised cDNA was amplified with internal primer RO339 (5' -TTG GAG AGG AGG AAG CGA CCA CCG AAG ATG ATG- 3') and a vector forward primer RO317 (5'- CAC ACA GGA AAC AGC TAT GAC CAT GAT TAC G -3'). Polymerase Chain Reaction (PCR) was carried out in a 100 µl volume containing: 300 ng of excised M. alpina cDNA library, 50 pmole each primer, 10 µl of 10X buffer, 1 µl 10 mM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 1.0 U of Taq Polymerase. Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94°C for 2 mins., then 30 cycles of 94°C for 1 min., 58°C for 2 mins., and 72°C for 3 mins. PCR was followed by an additional extension at 72°C for 7 minutes.

The PCR amplified product was run on a gel, an amplified fragment of approximately 360 bp was gel purified, and the isolated fragment was directly sequenced using ABI 373A DNA Sequencer (Perkin Elmer, Foster City, CA). The sequence analysis package of GCG was used to compare the obtained sequence with known sequences. The sequence was translated in all six reading frames in the GCG Analysis Program using the FastA algorithm (Pearson and Lipman, supra). The Swissprot

database (GeneBio, Geneva, Switzerland) of proteins was searched. This translated cDNA fragment was identified as a part of a putative elongase based on the homology of the putative protein sequence to the S. cerevisiae ELO2 (GNS1), having 41.3% identity in 63 amino acids.

New primers were designed based on the putative elongase sequence and the vector, pZL1 (Life Technologies, Inc., Gaithersburg, MD) sequence used to construct M. alpina cDNA library. The M. alpina excised cDNA library was PCR amplified again using primers RO350 (5' -CAT CTC ATG GAT CCG CCA TGG CCG CCG CAA TCT TG- 3'), which has an added *BamHI* restriction site (underlined), and the vector reverse primer RO352 (5' -ACG CGT ACG TAA AGC TTG- 3') to isolate the full length M. alpina elongase cDNA, using previously described conditions. The termini of the approximately 1.5 Kb PCR amplified fragment was filled-in with T4 DNA polymerase (Boehringer Mannheim Corp., Indianapolis, IN) to create blunt ends and cloned into the pCR-blunt vector (Invitrogen Corp., Carlsbad, CA). This resulted in two clones, pRAE-1 and pRAE-2 (see Figure 4A). (Plasmid DNA pRAE-2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, on August 28, 1998, under the terms of the Budapest Treaty, and was accorded deposit number ATCC 203166.) The elongase cDNAs from these vectors were cut out as an *EcoRI* fragment and cloned into the *EcoRI* digested pYX242 (Novagen, Madison, WI) vector. The clones pRAE-5 and pRAE-6 (see Figure 4B) have the elongase cDNAs from pRAE-1 and pRAE-2, respectively. (Plasmid DNA pRAE-5 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on August 28, 1998, under the terms of the Budapest Treaty, and was accorded deposit number ATCC 203167.) The sequencing of pRAE-5 and pRAE-6 revealed that 5' untranslated region of the

elongase gene in pRAE-5 is 16 bp shorter than that in pRAE-6 (see Figure 5). The complete M. alpina elongase cDNA sequence, designated MAELO was obtained from pRAE-2 (see Figure 6). Figure 7 is the amino acid sequence obtained from the translation of MAELO. The Swissprot database (GeneBio, Geneva, Switzerland) was searched again, as previously described, with the translated MAELO: MAELO has 44.3% identity in 317 amino acids with S. cerevisiae GNS1(ELO2) and 44.7% identity in 318 amino acids with S. cerevisiae SUR4(ELO3). The FastA alignment among the three elongases is shown in Figure 8. At the nucleotide level (see Figure 9), MAELO has 57.4% identity in 549 bp overlap with S. cerevisiae GNS1(ELO2) (GenBank Accession # S78624). However, the identity between the complete MAELO gene of 954 bp and S. cerevisiae GNS1(ELO2) is 33.0%.

Example III

Expression of *M. alpina* Elongase cDNA in Baker's Yeast

The constructs pRAE-5, and pRAE-6 were transformed into S. cerevisiae 334 (Hoveland et al., Gene 83:57-64 (1989)) and screened for elongase activity. The plasmid pCGN7875 (Calgene LLC, Davis, CA) containing jojoba KCS gene in pYES2 vector (Invitrogen Corp., Carlsbad, CA) was used as a positive control. The substrate used to detect elongase activity in M. alpina elongase (MAELO) was GLA and that in jojoba KCS was oleic acid (OA). The negative control strain was S. cerevisiae 334 containing pYX242 vector. The cultures were grown for 40-48 hours at 25°C, in selective media (Ausubel *et al.*, Short Protocols in Molecular Biology, Ch. 13, p. 3-5 (1992)), in the presence of a particular substrate. The expression of the jojoba KCS gene cloned in pYES2 was under the control of GAL1 promoter, while the promoter in pYX242 is

TP1, which is constitutive. Hence, the 334(pCGN7875) and 334(pYES2) cultures were induced with galactose. The GC-FAME analysis of the lipid fraction of each cell pellet was performed as previously described (Knutzon et al., supra).

The elongase activity results from different experiments are provided in Figure 10A and 10B. The jojoba KCS elongates long chain monounsaturated fatty acids 18:1n-9 to 20:1n-9. The amino acid homology between the M. alpina elongase (MAELO) and the S. cerevisiae ELO2 and ELO3 suggested that the proteins encoded by these genes may have similar substrate specificity. The activity of the M. alpina elongase, elongation (MAELO) of long chain monounsaturated and saturated fatty acids, is seen in the conversion of 18:1n-9 to 20:1n-9 and also in the synthesis of 24:0. The control strain, 334(pYX242) has very little or no detectable amount of 20:1 and 24:0 (see Figure 10A). M. alpina elongase (MAELO) also acts on at least one PUFA, converting 18:3n-6(GLA) to 20:3n-6(DGLA). The percentage of the 20:3n-6 in total lipid is higher in the strain 334(pRAE-5) and 334(pRAE-6) with the M. alpina elongase (MAELO) cDNA when compared to that in the control 334(pYX242). The percentages of 20:3n-6 produced were 0.092% for 334(pYX242) vs. 0.324% for 334(pRAE-5) and 0.269% for 334(pRAE-6) (shown in parenthesis in Figures 10A and 10B). This difference in the fatty acid profile is also seen in the total amount of 20:3n-6 produced. Only 0.226 μg of 20:3n-6 was produced by 334(pYX242) while 334(pRAE-5) and 334(pRAE-6) produced 2.504 μg of 20:3n-6 and 1.006 μg of 20:3n-6, respectively. Also, when no substrate is added, the level of 20:3n-6 is not detectable.

Once 20:3n-6 is generated by the M. alpina elongase (MAELO), the Δ5-desaturase can convert it to AA in the desired expression system. To test this hypothesis, the constructs pRAE-5 and pCGR-4 (a Δ5-desaturase containing

plasmid) were co-transformed into S. cerevisiae 334 and screened for AA production. The substrate used was 25 μM GLA (18:3n-6). If the M. alpina elongase (MAELO) is active in yeast, then the substrate will be converted to DGLA(20:3n-6), which the Δ5-desaturase will convert to AA(20:4n-6). The results in Figure 11 confirm the production of AA and therefore, the activity of the M. alpina elongase (MAELO).

The expression of Δ5-, Δ6-, and Δ12-desaturases, in yeast, along with the elongase, should result in the production of AA (see Figure 1) without the need for an exogenous supply of fatty acids.

Example IV

A Comparison of the Expression of M. alpina Elongase cDNA MAELO and S. cerevisiae Elongase ELO2 in Baker's Yeast

The ELO2 gene encoding for the yeast elongase was cloned from an S. cerevisiae genomic library (Origene, Rockville, MD) using the primers RO514 (5' -GGC TAT GGA TCC ATG AAT TCA CTC GTT ACT CAA TAT G-3') and RO515 (5' -CCT GCC AAG CTT TTA CCT TTT TCT TCT GTG TTG AG-3') incorporating the restriction sites (underlined) *BamHI* and *Hind*III (respectively). The ELO2 gene was cloned into the vector pYX242 at the *BamHI* and *Hind*III sites, designated pRELO, transformed into the S. cerevisiae host 334 (Hoveland et al., supra) and screened for PUFA elongase activity. The vector plasmid was used as a negative control and 334(pRAE-5) was grown to compare the PUFA elongase activity. The cultures were grown as previously described with no galactose in the media and 25 μM GLA added as a substrate. Figure 12 shows that amount of 20:3n-6 or DGLA produced (elongated from 18:3n-6 or GLA) by 334(pRAE-5) was approximately 4 times the negative control containing the unaltered vector pYX242, while the two individual clones 334(pRELO-1) and 334(pRELO-2) were only

twice the negative control. Additionally, when DGLA produced is expressed as a percent of the total lipids (shown in parenthesis, Figure 12), the clones 334(pRELO-1) and 334 (pRELO-2) produced 0.153% and 0.2% DGLA respectively, while 334(pYX242) produced 0.185% DGLA. Hence all these strains produced comparable percentages of DGLA. The strain 334(pRAE-5), however, produced 0.279% DGLA, an increase of 50.8% over 334(pYX242) (negative control). These data show that the S. cerevisiae elongase gene ELO2, even when overexpressed in yeast, does not elongate GLA to DGLA effectively. The M. alpina PUFA elongase activity is specific for this conversion as evidenced by the higher amount of DGLA produced compared to the control, 334(pYX242).

Example V

Identification of Elongases from Other Sources Using MAELO

The TFastA algorithm (Pearson and Lipman, supra) is used to search for similarity between a query peptide sequence and the database DNA sequence translated in each of the six reading frames. Translated MAELO was used as the query for a TFastA search in GCG with the GenEMBL database (6/98) from GCG to identify other potential elongase sequences based on their amino acid similarity comparisons to translated MAELO. For example, in Figures 13 and 14, two alignments are shown between translations of two different C. elegans sequences from chromosome III and MAELO. C. elegans DNA sequence (GenBank accession # Z68749) was annotated denoting similarity with GNS1 (ELO2), while the additional C. elegans DNA sequence (GenBank accession # U61954) was noted as similar to both GNS1 and SUR4 (ELO3). These are spliced DNA fragments in which the introns have been removed from the genomic sequence, and the exons assembled and translated.

The amount of amino acid identity between the putative PUFA elongases from C. elegans and translated MAELO are around 30%. This would point towards a common function in the fatty acid metabolism, e. g., a PUFA elongase. Figure 15 is another example of a translated C. elegans sequence (GenBank accession # AF003134) from chromosome III. The DNA sequence was identified that had DNA homology to the S. cerevisiae ELO2. Further inspection of this DNA sequence and its amino acid translation determined that there was homology to translated MAELO. C. elegans, therefore, may contain a PUFA elongase.

Figure 16 shows the alignments of translated DNA sequences from mouse and human, respectively, with translated MAELO. The mouse sequence CIG30, GenBank accession # U97107, was isolated from brown adipose tissue and reported as being "similar to yeast SUR4 protein". As shown in Figure 16, amino acids numbered 130 to 152 in the U97107 translation contain a high degree of similarity to the translated MAELO. The human sequence, GenBank accession # AC004050, from chromosome 4 was from an HTGS (High Throughput Genome Sequence). There were no annotations contained with this sequence. However, translated AC004050 had 28.7% identity in 150 amino acids with translated MAELO. This gene fragment could be a fragment of a human PUFA elongase based on its amino acid similarity to translated MAELO.

Figure 17 shows the amino acid alignment of translated MAELO and a mammalian sequence (GenBank accession # I05465, PCT# WO 88/ 07577) which claims that the protein derived from expression of this sequence is a glycoslylation inhibition factor. The amino acid identities between the two proteins, signifying that there could be related function, such as PUFA elongase activity.

These examples of other translated DNA sequences and their homology to the translated MAELO illustrate that any of the above examples could potentially be a PUFA elongase. These examples are not inclusive of all the possible elongases. However, use of MAELO or its amino acid translation as a query for database searches can identify other genes which have PUFA elongase activities.

Example VI

M. alpina cDNA Library Screening Using A Plaque Hybridization Method

In an effort to isolate additional PUFA elongase genes from M. alpina, a conventional plaque hybridization method was used to screen an M. alpina cDNA library made in a lambda vector. The DNA probe was generated based on MAELO nucleotide sequence and was used to screen the M7+8 M. alpina cDNA library made in a λZiplox vector (Knutzon et al., J. Biol. Chem. 273:29360-29366 (1998)).

To make the DNA probe for screening the library, the MAELO cDNA was digested with *Nsp*I and *Pvu*I restriction endonucleases. Three small DNA fragments, with an average size of approximately 300 bp, were produced and used as probes. The rationale for using a mixture of fragmented MAELO cDNA was based on the assumption that there might be a common region or domain in the amino acid sequence which is conserved among various PUFA elongases present in M. alpina. Using MAELO DNA probes, the cDNA library was screened by a plaque hybridization technique according to standard protocol (Sambrook et al., Molecular Cloning, 2nd Ed., Cold Spring Harbor, 1989).

Briefly, 50,000 primary clones were plated and transferred to nylon membranes. The membranes were denatured

and hybridized with alpha ^{32}P-dCTP-labelled MAELO DNA probes overnight in the hybridization buffer which contained 20% formamide, 0.2% PVP, BSA, Ficoll, 0.1% SDS and 0.5 M NaCl. The filters were washed with 0.5X SSC at 37 °C and exposed to X-ray film for autoradiography. This procedure was repeated three times. Four clones (designated as F1, F2, F3, and F4) which hybridized repeatedly were picked and suspended in SM buffer (Sambrook et al., supra) containing 7% DMSO.

The largest open reading frame of each candidate was subcloned into yeast expression vector pYX242 (Novagen, Inc., Madison, Wisconsin). The cDNA clones F1 and F3 were subcloned into pYX242 at the *EcoRI* site while F2 and F4 were subcloned at *NcoI*/*HindIII* sites. The recombinant pYX242 containing each candidate was transformed into SC334 (Hoveland et al., supra) for expression in yeast. To determine the elongase activity, as well as substrate specificity, SC334 containing each cDNA clone was grown in minimal media lacking leucine in the presence of 25 μM of GLA substrate as described in Example III. The fatty acid analysis was performed as described in Knutzon et al. (J. Biol. Chem. 273:29360-29366 (1998)). The results indicated that none of these four cDNA clones showed any significant activity in converting GLA to DGLA. Thus, the hybridization approach appeared to be unsuccessful in identifying additional PUFA elongases.

Example VII

Construction of Direct cDNA Expression Library of M. alpina in Yeast

To identify PUFA elongase genes other than MAELO, a different approach was taken to screen the M. alpina cDNA library. In particular, since Baker's yeast is incapable of

producing long chain PUFAs due to the absence of respective desaturases and elongases, an attempt was made to construct an expression cDNA library of M. alpina in Saccharomyces cerevisiae. The vector pYES2 (Novagen, Inc., Madison, Wisconsin), containing the GAL1 promoter, was chosen for the expression of cDNA library in S. cerevisiae.

The conventional way by which a cDNA library is made (i.e. transformation of cDNA/vector ligated DNA mixture into host cells) is difficult in yeast because the transformation efficiency by direct electroporation of ligated DNA mix is very low compared to the efficiency of purified supercoiled plasmid DNA. However, the major advantage of this method is to avoid amplification of primary clones which happens when the library is made in E. coli as an intermediate. Due to the limitation in the number of colonies to be screened, it was decided to first optimize the efficiency of transformation in different S. cerevisiae strains using cDNA/vector ligated mix. The best results were obtained with a yield of 4-5 x 10^5 transformants per μg of ligated DNA in S. cerevisiae strain SC334 (Hoveland et al., supra).

To make a direct M. alpina cDNA expression library in yeast total RNA was isolated from the fungus. M. alpina fungus (ATCC # 32221) was plated onto cornmeal agar (Difco Laboratories, Detroit, MI) and grown at room temperature for 3-4 days. Once fungus growth was visible, it was inoculated into 50 ml of potato dextrose broth and shaken at room temperature very slowly to formulate spores. Once spores were visible, the 50 ml culture was inoculated into a 1 liter culture of potato dextrose, and spores were grown for 72 hours. After filtering through sterile gauze, the cells were immediately frozen into liquid nitrogen for future RNA extraction. Total RNA was prepared from 36 g of cell pellet using the hot phenol/LiCl extraction method (Sambrook et al.,

supra). The cell pellets were homogenized in a 10 mM EDTA, 1% SDS and 200 mM sodium acetate, pH 4.8 solution. Phenol and chloroform were added to the homogenates, and the aqueous layer was extracted. The aqueous layer was back extracted one more time with phenol and chloroform. Then an equal volume of 4 M lithium chloride was added. The samples were ethanol precipitated on ice for 3 hours, and pellets were obtained by centrifugation. The RNA pellets were washed with 70% ethanol and resuspended in DEPC treated water. Total RNA was quantitated by spectrophotometry and visualized by agarose gel electrophoresis to confirm the presence of 28S and 18S ribosomal bands. Approximately, 15 mg of total RNA were obtained from 36 gram of cell pellet.

The library was constructed according to the standard protocol (Sambrook et. al., Molecular Cloning, 2nd Ed.. Cold Spring Harbor, 1989). Messenger RNA was prepared from the total RNA using oligo dT cellulose affinity purification. Messenger RNA was reverse transcribed with oligo dT primer containing a *Xho*I restriction site using AMV reverse transcriptase. Following first strand cDNA synthesis, the second strand of cDNA was synthesized by adding E. coli DNA polymerase, E. coli DNA ligase and RNAse H.

The *EcoR*I adaptor was ligated into the blunt-ended cDNA by T4 DNA ligase. The cDNA sample was kinased using T4 polynucleotide kinase and digested with *Xho*I, diluted with column buffer and passed through a Sephacryl S-400 column. The DNA samples were eluted by high salt buffer. Samples containing DNA from 400-5,000 bps were pooled and used for ligation into a pYES2 vector (Invitrogen Corp., Carlsbad, CA). The cDNA was ligated into the *EcoR*I/*Xho*I digested pYES2 vector using T4 DNA ligase. A large scale ligation reaction was carried out since a large amount of the ligated DNA (2-3 μg) is required in direct transformation of yeast.

To transform yeast cells directly with the cDNA/pYES2 ligated mixture, competent SC334 cells were prepared using the LiAc TRAFO method (Gietz et. al., Mol. Cell. Biol, 5: 255-269, 1995). Briefly, fresh culture of SC334 from the plate was inoculated into 50 ml YPD medium. The culture was grown at 30 °C with shaking until the OD at 600 had reached 1.0. Thirty ml of this starter was inoculated into 300 ml of YPD liquid medium and incubated with shaking until the cell number of the culture reached ~ 3-5 x 10^6 cell/ml (approximately 3-4 h). The cells were harvested and washed with sterile water. The entire cell pellet was resuspended in 1.5 ml of freshly prepared 1X TE/LiAc (0.1M LiAc). These cells were used immediately for the transformations.

Seven hundred and fifty microliters of competent SC334 cells were aliquoted into 15 ml falcon tubes. Approximately 2 ug of cDNA/pYES2 ligated DNA were added to the cells along with carrier DNA and mixed gently. Three milliliters of sterile 40% PEG/LiAc was added to the cells and mixed gently but thoroughly. The cells were incubated at 30 °C for 30 min with shaking and subsequently given heat shock at 42 °C for 15 min. The cells were cooled, pelleted, and resuspended in 5 ml of 1X TE. A 100 ul aliquot of the above cells was plated onto fifty 150 mm selective agar plates lacking uracil (Ausubel et al., supra) and incubated at 30 °C for 3 days. A total of 8 x 10^5 primary clones were obtained. Five colonies were pooled in 1 ml minimal media lacking uracil (Ausubel et al., supra) and glycerol added to prepare stocks. A total of 5,000 pools were made for screening.

Example VIII

MAD (M. alpina Direct) Screening in Yeast

The quality of the library was analyzed by determining the average size of the cDNAs in the library. Since the screening of the library was based on the expression of the cDNA, it was important to determine the average size of the cDNA present in the library. The expression library containing the longest cDNAs would be the best appropriate choice to isolate full-length cDNAs of interest. To this end, randomly selected pools were plated onto selective agar plates, as described in Example VII, to obtain individual colonies. Forty different yeast colonies were randomly picked, and each colony was inoculated into 5 ml of selective liquid medium lacking uracil (as described in Example VII) and grown, while shaking, for 24 hours at 30 ^{0}C. Plasmid DNA was extracted from these colonies by the bead beating method (Hoffman et al., Gene 57:267 (1987)) adapted as follows:

Pellets from 5 ml of culture were lysed in 0.5 ml of a 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA and 0.1% SDS solution. Sterile 0.5 mm glass beads of equal volume were added and manually vortexed for 3 minutes. Two hundred microliters of the same buffer were added, and the mixture was vortexed for an additional minute. The samples were centrifuged on high for 2 minutes, and cytoplasmic extract was then transferred to a fresh tube. An equal volume of phenol/$CHCl_3$ was added to the sample, vortexed and centrifuged again for 2 minutes. The aqueous layer was re-extracted twice and precipitated with 0.3 M sodium acetate and approximately 2.5 volumes of ethanol for 30 minutes at -20 ^{0}C. The precipitates were washed with 70% ethanol and resuspended in water. To eliminate RNA and any protein contamination, the plasmid DNAs isolated from 40 different samples were

further purified using the QIAprep Spin Miniprep Kit according to the manufacturer's protocol (Qiagen Inc., Valencia, CA). The plasmid DNA samples were then restricted with *EcoRI* and *XhoI* restriction endonucleases to release the cDNA fragment, and the digest was analyzed on 1% agarose gel. The results indicated that the majority of the cDNAs of the direct library varied in length from 0.8 Kb to 1.5 Kb.

To screen the library, the glycerol stocks were thawed and approximately 0.5 ml was added to 5 ml of liquid selective media lacking uracil (Ausubel et al., supra) and grown at 30°C for 24 hours. The culture was then transferred into 50 ml of liquid selective medium lacking uracil with 2% galactose and 25 μM GLA (substrate for the elongase enzyme) for 24 hours at 25 °C with shaking. The GC-FAME analysis of the lipid content in the cell pellet of each induced culture was performed as previously described (Knutzon et al., supra). The MAELO (pRAE-5 in pYX242 grown in selective media lacking leucine) was used as a positive control in each batch run. MAELO had consistently been able to convert 1.5% of GLA to DGLA (see Example III).

Example IX

Identification of a cDNA Encoding a Potential PUFA Elongase

After screening and analyzing approximately 750 individual pools by GC-FAME analysis, as described in Example VIII, one pool of five colonies (i.e., MAD708) appeared to have significant enzymatic activity in converting GLA to DGLA. This activity was found to be approximately 5 fold higher than the M. alpina elongase activity (MAELO) in terms of DGLA/GLA ratio (Figure 19). This pool was tested again under identical assay conditions to confirm the initial

findings. The repeat experiment showed 9.5% conversion of GLA to DGLA and was again around 5 fold higher than M. alpina elongase activity (MAELO). These results strongly indicated that the MAD708 pool contained an elongase candidate which was specific for GLA as substrate. Since MAD708 was a pool of five different clones, it was necessary to isolate the individual cDNA clone which encoded for elongase activity from this pool. To do this, the original MAD708 glycerol stock was plated onto a selective media agar plate lacking uracil (Ausubel et al., supra). Thirty individual colonies were picked and grown in liquid selective medium, lacking uracil with 2% galactose, as previously described in Example VIII, in the presence of GLA. The cell pellet obtained from each culture was then subjected to fatty GC-FAME analysis (Knutzon et al., supra) along with a positive control of 334 (pRAE-5)(MAELO in pYX242). The fatty acid analysis from the 30 individual clones from the MAD708 expression pool in yeast revealed that 5 of the 30 clones showed elongase activity in converting GLA to DGLA. The fatty acid profiles of the active clones MAD708-2, MAD708-10, MAD708-18, MAD708-19 and MAD708-30 are shown in Figure 20. As shown in this Figure, MAD708-2, 10, and 30 produced the most DGLA, approximately 25 fold more than MAELO (pRAE-5). These 3 converted in the range of 41% to 49% of GLA to DGLA. Other clones, MAD708-18 and MAD708-19, converted 8% and 21% of GLA to DGLA, respectively. All MAD708 clones converted a higher percentage of GLA to DGLA with respect to MAELO encoded elongase (3.4%).

Example X

Characterization of cDNAs Encoding Elongase

Plasmid DNA was extracted from SC334 yeast clones (MAD708 pool) that showed significant GLA specific elongase activity by the bead beating method, as described in Example VIII. To determine the size of the cDNA insert, PCR was performed using each plasmid DNA obtained from positive elongase clones as a template. The forward primer RO541 (5'-GAC TAC TAG CAG CTG TAA TAC -3') and the reverse primer RO540 (5'- GTG AAT GTA AGC GTG ACA TAA -3') are in the multicloning site of the pYES2 vector and were used to amplify the cDNA insert within the *EcoR*I and *Xho*I sites. PCR reaction was performed in a 50 μl volume containing 4 μl of plasmid DNA, 50 pmole of each primer, 5 μl of 10X buffer, 1 μl 10 μM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 0.5 μl of High Five Taq polymerase (Boehringer Mannheim, Indianapolis, IN). The amplification was carried out as follows: 2 mins. denaturation at 94 °C, then 94 °C for 1 min, 55 °C for 2 mins., and 72 °C for 3 mins. for 30 cycles, and 7 mins. extension at 72 °C at the end of the amplification. Analysis of PCR amplified products on a 1% agarose gel showed the sizes of the elongase cDNAs to be around 1.0 - 1.2 Kb. The plasmid DNAs, containing the potential elongase cDNAs, were designated as pRPB2, pRPB10, pRPB18, pRPB19, and pRPB30. Since the cDNA library was made in the pYES2 vector at the *EcoR*I and *Xho*I sites, the size of the cDNA present in each plasmid was further confirmed by digesting the above plasmids with *EcoR*I and *Xho*I.

The plasmid DNAs isolated from yeast were re-amplified in E. coli for long-term storage of the cDNA clones as well as for DNA sequencing. E. coli TOP10 (Invitrogen Corp., Carlsbad, CA) cells were transformed with the pRPB recombinant plasmids according to the manufacturer's protocol. The transformants obtained from each plasmid DNA were inoculated into LB containing ampicillin (50 μg/ml) and

grown overnight at 37 °C with shaking. Plasmid DNAs were isolated from these cultures by using QIAprep Spin Miniprep (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. The purified plasmid DNAs were then used for sequencing from both 5' and 3' ends. The DNA sequencing was performed by using a 373A Stretch ABI automated DNA sequencer (Perkin Elmer, Foster City, CA) according to the manufacturer's protocol. Primers used for sequencing were the forward primer RO541 (5'- GAT TAC TAG CAG CTG TAA TAC - 3') and the reverse primer RO540 (5'- GTG AAT GTA AGC GTG ACA TAA -3') contained in the multicloning sites of the pYES2 vector. The obtained nucleotide sequences were transferred to Sequencher software program (Gene Codes Corporation, Ann Arbor, MI) for analysis. The DNA sequence analysis revealed that all five elongase cDNAs contained the identical nucleotide sequence with a common overlap of 301 nucleotides. Each DNA sequence contains a putative start site at the beginning of the 5' end and a stop codon with poly A tail at the end of the 3' site. To further confirm the DNA sequence, internal forward primers RO728 (5'- GAG ACT TTG AGC GGT TCG -3') and RO730 (5'- TCT CTG CTG CGT TGA ACT CG -3'), along with reverse primers RO729 (5'- AAA GCT CTT GAC CTC GAA C -3') and RO731 (5'- AAC TTG ATG AAC GAC ACG TG -3') were designed within the cDNA, and used for sequencing of pRPB2, since this candidate possessed the highest elongase activity. The entire nucleotide sequence was analyzed by the Sequencher program (Figure 21), and the longest open reading frame deduced from the entire cDNA sequence in pRPB2 appeared to be 957 bp in length (Figure 22). The deduced open reading frame was then translated into the corresponding amino acid sequence, and the predicted sequence is shown in Figure 23. The elongase encoded by the cDNA (pRPB2) identified from M. alpina appears to be a 318 amino acid long protein which is

nearly identical in size with translated MAELO. This new elongase cDNA was designated as "GLELO" and its encoded protein has been named "GLA elongase".

Plasmid DNA pRPB2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 on July 22, 1999 under the terms of the Budapest Treaty. It was accorded ATCC Deposit # PTA-402.

Example XI

Biochemical Characterization of GLA Elongase (GLELO)

A. Confirmation of GLA Elongase Activity

To further confirm the activity of the GLA elongase encoded by the pRPB2 recombinant plasmid, elongase activity screening was repeated on the yeast clone SC334 containing pRPB2 plasmid. This experiment was also conducted to assure consistent lipid extraction and to detect the activity of GLA elongase by averaging four independent experiments. The S. cerevisiae 334 glycerol stock containing pRPB2 was plated onto minimal media agar plates lacking uracil. Individual colonies were randomly picked and grown in minimal medium lacking uracil, as described in Example VIII. The four independent cultures were combined, and a 5 ml aliquot was used as an inoculum for four separate 50 ml cultures. The cultures were then grown in the presence of GLA and were subjected to fatty acid analysis along with a negative control of S. cerevisiae 334 containing pYES2, as described in Example VIII. The average elongase activity from four independent cultures of 334(pRPB2) with 25 μM GLA is shown in Figure 24. The GLA elongase activity of each of the four independent samples of 334(pRPB2) appeared to be consistent with an average conversion of 62% GLA to DGLA.

B. Determination of GLELO Substrate Specificity for GLA Elongase

To analyze the substrate specificity of the GLA elongase, the culture of 334(pRPB2) was tested with different fatty acid substrates besides GLA (e.g., SA(18:0), OA(18:1), LA(18:2n-6), AA(20:4n-6), ADA(22:4n-6), ALA(18:3n-3), STA(18:4n-3), and EPA(20:5n-3)). Under identical assay conditions, the only other substrate utilized by the elongase enzyme was STA, a fatty acid from the n-3 pathway. GLA elongase was able to convert 73% of STA to 20:4n-3 (Figure 25). From these experiments, it can be concluded that the GLA elongase has substrate specificity for both GLA and STA, indicating that it possesses elongase activity along both the n-6 and n-3 pathways.

C. Co-expression of Fungal GLELO and Δ5-Desaturase Gene in Yeast

Once DGLA (20:3n-6) is produced by the GLA elongase, the Δ5-desaturase can convert it to AA (20:4n-6) in a desired co-expression system. This scheme, as depicted in Figure 1, can be tested by co-transforming S. cerevisiae 334 with plasmids pRPB2 and pRPE31 (the recombinant plasmid pYX242 containing a Δ5-desaturase cDNA (Figure 18) cloned at the *EcoRI* site. The co-transformed yeast cultures were supplemented with 25μM GLA and analyzed for AA synthesis. If both elongase and Δ5-desaturase enzymes are expressed, the GLA substrate will be converted to DGLA, which will then be converted to AA. The results in Figure 26A indicate that the sequential action of GLA elongase and Δ5-desaturase on GLA substrate resulted in an average conversion of 27% GLA to AA. Therefore, the GLA elongase has the ability to work with other enzymes in the n-6 PUFA synthetic pathway to produce desirable fatty acids.

To determine whether the above conversion is also true in n-3 pathways, the similar co-expression experiments were carried out in the presence of 25 μM STA. Again, if both enzymes are expressed, the STA substrate will be converted to 20:4n-3 which will then be converted to EPA (20:5n-3) by the Δ5-desaturase. Figure 26B shows the results in which the production of EPA (approx. 40%) is observed. Once again, the GLA elongase demonstrates its ability to work with Δ5-desaturase in the n-3 pathway to produce desirable fatty acids.

Example XII

Sequence Comparison Between GLELO and Other Fungal Elongases

The sequence analysis package of GCG (see Example I) was used to compare the GLELO sequence with known protein sequences. The nucleotide sequence of GLELO open reading frame was first translated into amino acid sequence that was used as a query sequence to search Swissprot database (see Example I) using the FastA algorithm (see Example I). Based on amino acid sequence similarity, the best matches were found with S. cerevisiae YJT6 (an EST with unknown annotation) with 33.9% identity in 189 amino acid overlap, S. cerevisiae ELO2 (GNS1) with 25.8% identity in 295 amino acid overlap, and S. cerevisiae ELO3 (SUR4) with 25.2% identity in 313 amino acid overlap. The FastA alignment of GLELO with MAELO showed 30.9% identity in 275 amino acids (Figure 27). GCG Pileup program creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments (see Example I), and was used with the elongases described above. The Pileup results indicate that there are many conserved regions among the elongases including a putative histidine box, which is underlined (Knutzon et. al., J. Biol. Chem. 273: 29360-29366,

1998) (Figure 28). Thus, although GLELO has similarity with MAELO, the difference in their encoded elongases may presumably be due to their substrate preference. GLA elongase can convert a higher percentage of GLA to DGLA than M. alpina elongase. In addition, MAELO expression in S. cerevisiae showed elongation of saturated and monounsaturated fatty acids in addition to GLA elongation to DGLA (see Example III).

Example XIII

Identification of M. alpina MAELO Homologues in Mammals

The MAELO translated sequence was used to search the Unified Human Transcript Database of Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, Illinois 60064. This database was searched using Basic Local Alignment Search Tool (BLAST) (Altschul et al., Nuc. Acids Res. 25:3389-3402 (1997)) which "is a set of similarity search programs designed to explore all of the available sequence databases regardless of whether the query is a protein or DNA." Specifically, the tblastn algorithm was used (i.e., a protein query search to a nucleotide database translated in six reading frames). The contig (CC) sequences in the Unified Human Transcript Database are consensus sequences representing groups of expressed sequence tags (EST) cDNAs derived from the public domain and from the Incyte LIFESEQ™ database of ESTs (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304) that are clustered together on the basis of defined sequence homology, and assembled on the basis of sequence overlap. Two sequences from this database, CC067284R1 and CC1484548T1 had 28% identity in 242 amino acid overlap and 28.6% identity in 266 amino acid overlap, respectively, with the translated MAELO sequence. The two derived and edited sequences were designated as hs1 and hs2, respectively, and

copied into the sequence analysis software package of GCG (see Example I). The translated MAELO sequence was aligned with translated HS1 (28.5% identity in 242 amino acids) and HS2 (28.2% identity in 266 amino acids) cDNA sequences using the FastA algorithm, as shown in Figures 29 and 30, respectively. HS1 cDNA nucleotide sequence also had 86.9% identity in 844 bp with the I05465 nucleotide sequence (see Example V). The translated HS2 cDNA sequence had 100% identity with the amino acid sequence from GenBank with accession number W74824 (see published PCT application WO9839448).

The National Center for Biotechnology Information (NCBI at http://www.ncbi.nlm.nih.gov/) was used to conduct database searches using tblastn with the 28 amino acid sequence (DTIFIILRKQKLIFLHWYHHITVLLYSW) translated from AC004050 (a human sequence identified in a TFastA search, see Example V). This amino acid sequence contains a histidine box (underlined), which has a noted motif of desaturases (Knutzon et al., supra), and both PUFA elongases, MAELO and GLELO (see Figure 28). A translated mouse sequence shown previously in Example V (GenBank Accession #U97107) and a translated C. elegans sequence (GenBank Accession # U41011) had the highest matches with this 28 amino acid query. The NCBI mouse EST database was searched again with tblastn, using translated U41011 as a query. An additional mouse sequence was identified (GenBank Accession #AF014033.1), annotated as "putative involvement in fatty acid elongation." Three longer sequences (GenBank Accession #'s AA591034, AA189549, and AA839346) were identified through a tblastn search of the mouse EST database with translated AF014033.1 and combined into one sequence designated as mm2. The FastA alignment (see Example I) of translated mm2 and MAELO is shown in Figure 31. Another related, but not identical mouse sequence

(GenBank Accession #AI225632), was also identified in a tblastn search of the mouse EST database with AF014033.1. The FastA alignment with translated AI225632 to MAELO is shown in Figure 32. The percent identity for both translated MM2 and AI 225632 with translated MAELO is 30.4% in 191 and 115 amino acid overlap, respectively. The level of amino acid identity with translated MAELO with these two translated mouse sequences identifies them as putative homologues of PUFA elongases.

Example XIV

Identification of M. alpina GLELO Homologues in Mammals

The TFastA algorithm, which compares a protein sequence to the database DNA sequence translated in each of the six reading frames, was used with translated GLELO as the query. The GenEMBL database from GCG was used to identify other potential elongase sequences based on their amino acid similarity to translated GLELO. Three human sequences were found to have matches with the GLELO amino acid sequence. These sequences have GenBank accession numbers 1) AI815960, 2) AL034374, and 3) AC004050. AI815960, a Homo sapien EST sequence, has 40.3% identity in 144 amino acid overlap with translated GLELO (see Figure 33). A translated region of the human genomic sequence AL034374, derived from chromosome VI has 46.7% identity in a 60 amino acid overlap with translated GLELO. This homologous region in AL034374 appeared to be a part of the HS1 amino acid sequence which was shown to have homology with translated MAELO (see Example XIII). Therefore, HS1 sequence has similarity with both MAELO (see Figure 29) as well as GLELO (see Figure 34). A translated region of a human genomic sequence AC004050 from chromosome IV has 34.8% identity in 89 amino acid overlap with translated GLELO (see Figure 35). The amino acid identities between GLELO and these human sequences indicate that the

proteins dervied from these human sequences could have related function, such as PUFA elongase activity.

To identify a mouse cDNA similar to GLELO, TFastA searches were performed with the GenEMBL database using translated GLELO as a query. From the TFastA searches, the three mouse sequences with the highest matches to translated GLELO were identified: (GenBank accession numbers 1) AF104033, 2) AI595258, and 3) U97107). AF104033 is annotated as "MUEL protein having putative fatty acid elongase with homology to yeast ELO3 (SUR4)" and is a part of the sequence of MM2. The MM2 sequence was initially derived from AF104033 mouse sequence, but the entire MM2 sequence was finally obtained through further mouse EST database searches and also shown to have homology with translated MAELO (see Example XIII and Figure 31). When this MM2 amino acid sequence was aligned with translated GLELO sequence using FastA, a 34.6% identity in 211 amino acid overlap was found (see Figure 36) indicating that MM2 also has homology with GLELO. AI595258 is a mouse cDNA clone having 5' similarity with yeast ELO3 elongase and is part of mouse EST cDNA AI225632. The AI225632 mouse sequence, which is a longer sequence than AI595258, was shown to have similarity with translated MAELO (see Figure 32). The AI225632 was also aligned with the translated GLELO, and the FastA alignment is shown in Figure 37. A 35.3% identity in 199 amino acid overlap has been found. The third sequence, U97107, a mouse sequence, was annotated as "similar to yeast ELO3 (SUR4) gene." The FastA alignment of translated GLELO with U97107 is shown in Figure 38 where a 23.7% identity in 279 amino acid overlap was found. Previously, a region of U97107 was also found to have a high degree of homology with MAELO based on a FastA alignment (see Example V and Figure 16).

The above searches clearly indicate that the same human and mouse sequences were obtained by using either MAELO or GLELO as a query.

Example XV

Identification of M. alpina GLELO and MAELO Homologues in Other PUFA Producing Organisms

A) Caenorhabditis elegans:

A putative amino acid sequence deduced from a chromosomal sequence of C. elegans (GenBank Accession # U41011) was able to identify a partial sequence contained in the mouse MM2 putative PUFA elongase which has amino acid similarity with both GLA elongase (GLELO) and M. alpina elongase (MAELO). It was therefore conceivable that C. elegans homologues of GLELO or MAELO might be present in the nematode database. The putative amino acid sequences derived from GLELO and MAELO sequences were used as queries independently to search the nematode databases. A BLAST search (see Example XIII) was performed on wormpep16 (blastp compares an amino acid query sequence against a nucleotide sequence database) and wormpep 16cDNAs (tblastn) databases which are predicted proteins and cDNAs obtained from the C. elegans genome sequencing project or EST's and their corresponding cDNA sequences, respectively. These sequence data were produced by the C. elegans Sequencing group, carried out jointly by the Sanger Centre and Genome Sequencing Center, and can be obtained from ftp://ftp.sanger.ac.uk/pub/ databases/wormpep/. At least seven putative C. elegans translated sequences were identified by their amino acid sequence homology to the translated amino acid sequence of both GLELO and MAELO. The GenBank Accession #'s of those genomic sequences containing

the deduced amino acids were identified as Z19154, U68749 (2 deduced proteins (F56H11.4 and F56H11.3 (wormpep Accession #'s)), U41011, U61954 (2 deduced proteins (F41H10.7 and F41H10.8 (wormpep Accession #'s)), and Z81058. Those underlined were identified in a previous search using translated MAELO as query (see Example V). As an example, the FastA amino acid alignments of translated U68749 (F56H11.4) with translated GLELO and MAELO are shown in Figures 39 and 40. Translated U68749 (F56H11.4) has 25-30% identity with both M. alpina elongase and GLA elongase in approximately a 200 amino acid overlap (see Figures 39 and 40). For all seven translated putative C. elegans cDNAs, the FastA alignments to translated GLELO was between 25-30% identity in a 200 amino acid overlap, while the identity was 26-34% in at least a 188 amino acid overlap for translated MAELO. The alignment similarities indicate that either translated GLELO or MAELO can be used to identify potential genes from C. elegans with elongase activity.

B) Drosophila melanogaster:

The translated deduced cDNA from the genomic sequence U41011 (C. elegans) had its highest match with a Drosophila melanogaster EST, accession number AI134173 in a blastn search (compares a nucleotide query sequence against a nucleotide database) of the "other ESTs" database through NCBI (see Example XIII) and was assembled with an overlapping DNA EST fragment, accession number AI517255. The translated DNA fragment DM1, derived from the two overlapping sequences was aligned with translated GLELO as well as MAELO (see Figures 41 and 42) using FastA in GCG (see Example I). The alignments showed 27.2% identity with GLA elongase in a 206 amino acid overlap and 30% identity with M. alpina elongase in a 237 amino acid overlap. Thus, based on amino acid

similarity, the DM1 could be a potential homologue to GLELO or MAELO having PUFA elongase-like activity. Moreover, using DNA sequences of GLELO and MAELO as queries for database searches, homologues with PUFA elongase activity from Drosophila can be identified.

Example XVI

Cloning and Expression of A Human PUFA Elongase Homologue

Many potential PUFA elongase sequences were identified based on their amino acid similarities to translated GLELO and/or MAELO. To determine the potential elongase activities of these sequences, the cDNA encoding the full length protein is then identified, cloned, and expressed, as demonstrated in the present example.

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 HS1 sequence, and used to amplify the human liver Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, California). The polymerase Chain Reaction (PCR) was carried out in a 50 μl volume containing: 5 μl of human liver Marathon-Ready cDNA, 50 pmole each primer, 1μl 10 mM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN), 5 μl 10 X buffer and 1.0 U of Advantage KlenTaq Polymerase Mix (Clontech Laboratories, Inc., Palo Alto, CA). Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins, and 72 °C for 3 mins. PCR was followed by an additional extension cycle at 72 °C for 7 minutes.

The PCR amplified product was run on a gel, an amplified fragment of approximately 960 bp was gel purified, the termini of the fragment filled-in with T4 DNA polymerase

(Boehringer Mannheim, Corp., Indianapolis, IN), and cloned into pCR-Blunt Vector (Invitrogen Corp., Carlsbad, CA) following manufacturer's protocol. The new plasmid was designated as pRAE-52, and the putative PUFA elongase cDNA in this clone was sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence in plasmid pRAE-52 is shown in Figure 43, and the translated sequence is shown in Figure 44.

The putative PUFA elongase cDNA from plasmid pRAE-52 was then digested with *NcoI/HindIII*, gel purified, and ligated into pYX242(*NcoI/HindIII*). The new plasmid was designated as pRAE-58-A1. (Plasmid 58-A1 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number ATCC PTA-566.)

The construct pRAE-58-A1 was transformed into S. cerevisiae 334 (Hoveland et al., supra) and screened for elongase activity. The negative control strain was S. cerevisiae 334 containing pYX242 vector. The cultures were grown for 24 hours at 30°C, in selective media (Ausubel et al., supra), in the presence of 25 μM of GLA or AA. In this study, DGLA or adrenic acid (ADA, 22:4n-6), respectively, was the predicted product of human elongase activity. When GLA was used as a substrate, the yeast cells containing the human elongase cDNA contained elevated levels of DGLA compared to control cells, 2.75% vs. 0.09% of total fatty acids, respectively (see Figure 45). When AA was used as a substrate, the yeast cells containing the human elongase cDNA contained elevated levels of ADA compared to control cells, none detected vs. 1.21% of total fatty acids, respectively. Thus, the human elongase converts both 18 and 20 carbon chain long PUFAs to their respective elongated fatty acids.

The yeast cells containing the human elongase cDNA also had elevated levels of monounsaturated fatty acids including 18:1n-7, 20:1n-7, 20:1n-9, and 18:1n-5, compared to the control strain. Therefore, these results indicate that the identified human elongase is capable of utilizing PUFAs as well as monounsaturated fatty acids as substrates. Thus, this human sequence HSELO1, and its encoded protein, possess elongase activity independent of substrate specificity.

Example XVII

Cloning and Expression of a C. elegans PUFA Elongase

Several putative C. elegans elongases were identified with amino acid homology to both translated GLELO and MAELO. As with the human cDNA sequence, cloning of a cDNA and expression in yeast was used to determine if indeed it was a PUFA elongase. Primers RO738 (5' -AAT CAG GAA TTC ATG GCT CAG CAT CCG CTC GTT CAA C -3') and RO739 (5' -CCG CTT GTC GAC TTA GTT GTT CTT CTT CTT TGG CAC -3') with restriction sites *EcoRI* and *SalI* (underlined), respectively, were based on the putative cDNA sequence contained in the genomic sequence U68749 (wormpep cDNA accession #F56H11.4.) A PCR amplification was performed in a 100 μl volume containing: 250 ng excised C. elegans library cDNA (OriGene Technologies Inc., Rockville, MD), 50 pmole each primer, 10 μl 10X reaction buffer (Boehringer Mannheim Corp., Indianapolis, IN), 1 μl 10 mM PCR Nucleotide mix (Boehringer Mannheim Corp., Indianapolis, IN), and 2.5 U Taq polymerase (Boehringer Mannheim Corp., Indianapolis, IN). Thermocycler conditions in a Perkin Elmer 9600 (Norwalk, CT) were as follows: 95 °C for 5 mins, then 25 cycles of 94 °C for 30 secs, 55 °C for 2 mins, and 72 °C for 2 mins. PCR was followed by an additional cycle of 72 °C for 7 minutes.

The PCR amplified product was purified from an agarose gel, cut with *EcoRI* and *SalI*, ligated to pYX242 (Invitrogen Corp., Carlsbad, CA) (linearized with *EcoRI* and *SalI*) using the Rapid Ligation kit (Boehringer Mannheim Corp., Indianapolis, IN), according to the manufacturer's protocol and transformed into E. coli Top10 cells (Invitrogen Corp., Carlsbad, CA). The new plasmids, designated pRET-21 and pRET-22 (two individual clones from the ligation), were sequenced with the 373A Stretch DNA sequencer ABI (Perkin Elmer, Foster City, CA), and the cDNA sequences were identical. The 867 base cDNA nucleotide sequence of the plasmid pRET-22 containing the putative elongase is shown in Figure 46 and the translated sequence of 288 amino acids is shown in Figure 47. (Plasmid pRET-22 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number ATCC PTA-565.)

The plasmids pRET-21 and -22 were transformed into S. cerevisiae 334 as previously described (see Example III) and the resulting yeast cultures (334(pRET-21) and 334(pRET-22)) grown in 100 ml of selective media without leucine (Ausubel et al, supra) for 48 hours at 20 °C in the presence of 50 μM GLA and AA. The cell pellets were collected and subjected to fatty acid analysis and the results shown in Figure 48. DGLA, the predicted product from GLA elongation, was found to be an average of 1.79% of the total lipid in the two samples, versus 0.13% for the negative control (334 containing plasmid pYX242) indicating that the enzyme encoded by both pRET-21 and pRET-22 possessed GLA elongase activity. The percent conversion of GLA to DGLA by 334(pRET-21) and 334(pRET-22) was 11.1% and 19.4% respectively with an average of 15.25%. Interestingly, almost no elongation of AA or any endogenous fatty acid was observed (Fig. 48). These results indicate

that the elongase encoded by this newly identified C. elegans cDNA, CEELO1, is able to specifically elongate GLA to DGLA, suggesting that it may be a C. elegans homologue of GLA elongase.

Example VIII

Isolation of a Putative Human Elongase cDNA Based on AC004050 Sequence

To isolate the full length putative elongase cDNA based on the AC004050 sequence, primers RP735 (5' -CCT CCT GAA TTC CAQA CAC TAT TCA GCT TTC -3') and R073 (5' -TAA TAC GAC TCA CTA TAG GG -3') were used to PCR amplify the human liver Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, CA). The PCR was carried out using the Advantage™ cDNA PCR Kit (Clontech Laboratories, Inc., Palo Alto, CA) with 5 μl of human liver Marathon-Ready cDNA and 50 pmole each primer following manufacturer's instructions. Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins., and 72 °C for 3 mins. PCR was followed by an additional extension at 72 °C for 7 mins.

The PCR amplified product was run on a gel, an amplified fragment of approximately 1 Kb was gel purified, the termini of the fragment were filled in with T4DNA polymerase (Boehringer Mannheim, Corp., Carlsbad, CA) following manufacturer's instructions. The new plasmid was designated as pRAE-59, and the putative PUFA elongase cDNA in this plasmid, designated as HS3, was sequenced using the ABI 373A Stretch Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence HS3 is shown in Figure 49, and the translated sequence is shown in Figure 50.

Nutritional Compositions

The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

Features:

-Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.

-Lactose-free formulation to avoid lactose-associated diarrhea.

-Low osmolality (240 mOs/kg water) to reduce risk of osmotic diarrhea.

-Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.

-1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

-Recommended levels of vitamins and minerals.

-Vegetable oils to provide recommended levels of essential fatty acids.

-Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0. 11 % calcium phosphate

tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and disglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

Features:
-First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
-Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
-Nutritionally complete to meet the nutritional needs of the infant.
-Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.
-Lactose-free formulation to avoid lactose-associated diarrhea.
-Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
-Dual carbohydrates (corn syrup and sucrose) designed to

enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
-Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
-1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
-Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve) 86% water, 4.8% com syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11 % calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

C. Isomil® SF Sucrose-Free Soy Formula With Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:

-Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.

-Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).

-Sucrose free for the patient who cannot tolerate sucrose.

-Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.

-1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

-Recommended levels of vitamins and minerals.

-Vegetable oils to provide recommended levels of essential fatty acids.

-Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 75% water, 11.8% hydrolized cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch, 0.38% calcium phosphate tribasic, 0. 17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, abscorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride,
carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate,L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

D. Isomil® 20 Soy Formula With Iron Ready To Feed, 20 Cal/fl oz.:

Usage: When a soy feeding is desired.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0. 11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, abscorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

E. Similac® Infant Formula:

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.

Features:

-Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.

-Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.

-Carbohydrate as lactose in proportion similar to that of human milk.

-Low renal solute load to minimize stress on developing organs.

-Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, abscorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

F. Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a nutritionally complete formula developed to provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

Features:

-Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).

-Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.

-Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.

-More calcium and phosphorus for improved bone mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.:

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: -D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine,

niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D3, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art.

II. NUTRITIONAL FORMULATIONS

A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

Patient Conditions:
-For patients on modified diets
-For elderly patients at nutrition risk
-For patients with involuntary weight loss
-For patients recovering from illness or surgery
-For patients who need a low-residue diet

Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic

Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B. ENSURE® BARS:

Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains gluten.)

Patient Conditions:
-For patients who need extra calories, protein, vitamins and minerals.
-Especially useful for people who do not take in enough calories and nutrients.
-For people who have the ability to chew and swallow
-Not to be used by anyone with a peanut allergy or any type of allergy to nuts.
Ingredients: Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy Polysaccharide, Glycerine, Whey Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa

Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes nuts.

Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein: Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

Soy protein isolate	74%
Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, oils, and soy lecithin.

Partially hydrogenated cottonseed and soybean oil	76%
Canola oil	8%
High-oleic safflower oil	8%
Corn oil	4%
Soy lecithin	4%

Carbohydrate: Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar,

maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

High-fructose corn syrup	24%
Brown sugar	21%
Maltodextrin	12%
Honey	11%
Crisp rice	9%
Glycerine	9%
Soy Polysaccharide	7%
Oat bran	7%

C. ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions:

-For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

Features:

-Low in saturated fat

-Contains 6 g of total fat and < 5 mg of cholesterol per serving
-Rich, creamy taste
-Excellent source of protein, calcium, and other essential vitamins and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-OIeic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Suffate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	85%
Soy protein isolate	15%

Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

High-oleic safflower oil	40%
Canola oil	30%
Soy oil	30%

The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Sucrose	60%
Maltodextrin	40%

Chocolate:

Sucrose	70%
Maltodextrin	30%

D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.
-For healthy adults who don't eat right and need extra nutrition.

Features:

-Low in fat and saturated fat
-Contains 3 g of total fat per serving and < 5 mg cholesterol
-Rich, creamy taste
-Excellent source of calcium and other essential vitamins and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients:

French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate

Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride), Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:
The protein source is calcium caseinate.

Calcium caseinate	100%

Fat:
The fat source is a blend of two oils: high-oleic safflower and canola.

High-oleic safflower oil	70%
Canola oil	30%

The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the, calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Sucrose	51%
Maltodextrin	49%

Chocolate:

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine:

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used

with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

Patient Conditions:
-For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
-For patients who need to gain or maintain healthy weight

Features:
-Rich, creamy taste
-Good source of essential vitamins and minerals

Ingredients:

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

The fat source is corn oil.

Corn oil	100%

Carbohydrate:

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, buffer pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla, strawberry, butter pecan, and coffee flavors:

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

Chocolate and eggnog flavors:

Corn Syrup	36%
Maltodextrin	34%
Sucrose	30%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

Patient Conditions:

-For patients with increased calorie and protein needs, such as following surgery or injury.

-For patients with limited volume tolerance and early satiety.

Features:

-For supplemental or total nutrition

-For oral or tube feeding

-1.5 CaVmL,

-High nitrogen

-Calorically dense

Ingredients:

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:
-For patients on modified diets
-For elderly patients at nutrition risk
-For patients recovering from illness/surgery
-For patients who need a low-residue diet

Features:
-Convenient, easy to mix
-Low in saturated fat

-Contains 9 g of total fat and < 5 mg of cholesterol per serving
-High in vitamins and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

The fat source is corn oil.

Corn oil	100%

Carbohydrate:

ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

Vanilla:

Corn Syrup	35%
Maltodextrin	35%
Sucrose	30%

H. ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

Patient Conditions:
-For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
-For patients with swallowing impairments

Features:
-Rich and creamy, good taste
-Good source of essential vitamins and minerals
-Convenient-needs no refrigeration
-Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:
Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is nonfat milk.

Nonfat milk	100%

Fat:

The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil	100%

Carbohydrate:

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors:

Sucrose	56%
Lactose	27%
Modified food starch	17%

Chocolate:

Sucrose	58%
Lactose	26%
Modified food starch	16%

I. ENSURE® WITH FIBER:

Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For patients who can benefit from increased dietary fiber and nutrients

Features:

-New advanced formula-low in saturated fat, higher in vitamins and minerals

-Contains 6 g of total fat and < 5 mg of cholesterol per serving

-Rich, creamy taste

-Good source of fiber
-Excellent source of essential vitamins and minerals
-For low-cholesterol diets
-Lactose- and gluten-free

Ingredients:
Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride, Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins-casein and soy.

Sodium and calcium caseinates	80%
Soy protein isolate	20%

Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

High-oleic safflower oil	40%
Canola oil	40%
Corn oil	20%

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of ≤ 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and ≤ 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Maltodextrin	66%
Sucrose	25%
Oat Fiber	7%
Soy Fiber	2%

Chocolate:

Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%

Soy Fiber 2%

Fiber:

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

J. Oxepa™ Nutritional Product

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil), γ-linolenic acid (GLA from borage oil), and elevated antioxidant levels.

Caloric Distribution:

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs. The distribution of Calories in Oxepa is shown in Table IV.

Table IV. Caloric Distribution of Oxepa

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)	25	105.5	28.1

Protein (g)	14.8	62.5	16.7
Water (g)	186	785	---

Fat:

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).
-The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table V.

-Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table VI.

-Medium-chain trigylcerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

Table V. Typical Fatty Acid Profile

	% Total Fatty Acids	g/8 fl oz*	9/L*
Caproic (6:0)	0.2	0.04	0.18
Caprylic (8:0)	14.69	3.1	13.07
Capric (10:0)	11.06	2.33	9.87
Palmitic (16:0)	5.59	1.18	4.98
Palmitoleic	1.82	0.38	1.62
Stearic	1.94	0.39	1.64
Oleic	24.44	5.16	21.75
Linoleic	16.28	3.44	14.49
α-Linolenic	3.47	0.73	3.09
γ-Linolenic	4.82	1.02	4.29
Eicosapentaenoic	5.11	1.08	4.55
n-3-Docosapent-aenoic	0.55	0.12	0.49
Docosahexaenoic	2.27	0.48	2.02
Others	7.55	1.52	6.72

Fatty acids equal approximately 95% of total fat.

Table VI. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz
	40.1 mg/L

Carbohydrate:

-The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).

-The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.

-The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (C02) production. High C02 levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.

-Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa

is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

-Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).

-The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.

-Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on C02 production, a high protein diet will increase ventilatory drive.

-The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.

- The amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.

* Oxepa is gluten-free.

Default settings for the analysis programs

GCG Programs

FastA Search

Default parameters:

range of interest	Begin=1	END=last protein or nucleic acid
search set	all of SwissProt (protein) or GenEMBL(nucleic acid)	
word size	=(2) for protein	=(6) for nucleic acid

Expected scores lists scores until E( ) value reaches 2.0

TFastA search

Default parameters:

range of interest	Begin=1	END=last nucleic acid
search set	all of GenEMBL	
word size	wordsize=(2)	

Expected scores lists scores until E() value reaches 2.0

Pileup

Default parameters:

gap creation penalty	gap weight = 5
gap extension penalty	gap length weight = 12
plot figure	one page plot density =2.7

Sequencher Program

Default parameters:

Automatic Assembly	Dirty data algorithm =slower contig assembly but more rigorous comparisons between the sequences

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minimum match =85%

minimum overlap =20

BLAST 2 (blastp, tblastn)

Default parameters:	V=50	Lambda=.329	W=3
	B=50	K=0.140	X=22
	E=10	H=0.427	

blast n

Default parameters:	V=100	Lambda=1.37	W=11
	B=250	K=0.171	X1=22
	E=10	H=1.31	X2=25

BLAST 2 Command Line Arguments

-v Hits number of best scores to show

-b Alignments number of best alignments to show

-e Expectation value (E) [Real] default = 10.0

-m Alignment view options: 0 = pairwise, 1 = master-slave showing identities, 2 = master-slave, no identities, 3 = flat master-slave, show identities, 4 = flat master-slave, no identities, 5 = master-slave, no identities and blunt ends, 6 = flat master-slave, no identities and blunt ends [Integer] default = 0

-F Filter query seq. (DUST with blastn, SEG with others) [T/F] default = T

-G Cost to open a gap (zero invokes default behavior) [Integer] default = 0

-E Cost to extend a gap (zero invokes default behavior) [Integer] default = 0

-X X dropoff value for gapped alignment (in bits) (zero invokes default behavior) [Integer]

```
                    default = 0

-I Show GI's in deflines                [T/F]
                    default = F

-q Penalty for a nucleotide mismatch    (blastn only) [Integer]
                    default = -3

-r Reward for a nucleotide match        (blastn only) [Integer]
                    default = 1

-f Threshold for extending hits         default if zero [Integer]
                    default = 0

-g Perfom gapped alignment (not available with tblastx) [T/F]
                    default = T

-q Query Genetic code to use            [Integer]
                    default = 1

-D DB Genetic code                      (for tblast[nx] only)
[Integer]
                    default = 1

-J Believe the query defline            [T/F]
                    default = F

-M Matrix                               [String]
                    default = BLOSUM62

-W Word size                            default if zero [Integer]
                    default = 0

-z Effective length of the database (use zero for the real size)
[Integer]
                    default = 0

-a Number of processors to use          [Integer]
                    default = site configurable (SeqServer.conf)

Allowed and default values for gap open/gap extension cost (-G/-E)
parameters:

BLOSUM62

-G          9   8   7   12  11  10
-E          2   2   2   1   1   1
BLOSUM50

-G          12  11  10  9   15  14  13  12  18  17  16  15
-E          3   3   3   3   2   2   2   2   1   1   1   1

PAM250
```

	-G	13	12	11	10	15	14	13	12	19	18	17	16
	-E	3	3	3	3	2	2	2	2	1	1	1	1
BLOSUM90													
	-G	8	7	6	11	10	9						
	-E	2	2	2	1	1	1						
PAM30													
	-G	5	4	3	7	6	5	10	9	8			
	-E	3	3	3	2	2	2	1	1	1			
PAM70													
	-G	6	5	4	8	7	6	11	10	9			
	-E	3	3	3	2	2	2	1	1	1			

The claims defining the invention are as follows:

1. An isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence shown in SEQ ID No: 1 (Figure 6), wherein said isolated nucleotide sequence encodes a polypeptide having elongase activity.

2. The isolated nucleotide sequence of claim 1 wherein said sequence is represented by SEQ ID NO: 1.

3. A purified protein encoded by said nucleotide sequence of claims 1 or 2.

4. A purified polypeptide which elongates polyunsatured fatty acids or monounsatured fatty acids and has at least about 50% amino acid similarity to the amino acid sequence of said purified protein of claim 3.

5. A method of producing an elongase enzyme comprising the steps of:

(a) isolating said nucleotide sequence represented by SEQ ID NO: 1 (Figure 6);

(b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;

(c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

6. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO: 1 (Figure 6) operably linked to b) a promoter.

7. A host cell comprising said vector of claim 6.

8. A plant cell, plant or plant tissue comprising said vector of claim 6, wherein expression of said nucleotide sequence of said vector results in production of at least one fatty acid selected from the group consisting of a monounsatured fatty acid and a polyunsatured fatty acid by said plant cell, plant or plant tissue.

9. One or more plant oils or fatty acids expressed by said plant cell, plant or plant tissue of claim 8.

10. A transgenic plant comprising said vector of claim 6, wherein expression of said nucleotide sequence of said vector results in production of a polyunsatured fatty acid in seeds of said transgenic plant.

11. A transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase operably linked to a promoter wherein said DNA sequence is represented by SEQ ID NO: 1 (Figure 6).

12. The transgenic, non-human mammal of claim 11 wherein said sequence encodes a functionally active elongases which utilizes a polysaturated fatty acid as a substrate.

13. A fluid produced by said transgenic, non-human mammal of claim 12 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

14. A method for producing a polyunsaturated fatty acid comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 6);

b) constructing a vector comprising said isolated nucleotide sequence;

c) introducing said vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by said isolated nucleotide sequence; and

d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

15. The method according to claim 14 further comprising the step of exposing said product polyunsaturated fatty acid to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

16. The method of claim 15 further comprising the step of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

17. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid

produced according to the method of claim 14, said another polyunsaturated fatty acid produced according to the method of claim 15, and said final polyunsaturated fatty acid produced according to the method of claim 16.

18. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 14, said another polyunsaturated fatty acid produced according to the method of claim 15, and said final polyunsaturated fatty acid produced according to the method of claim 16 and 2) a pharmaceutically acceptable carrier.

19. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 14, said another polyunsaturated fatty acid produced according to the method of claim 15 and said final polyunsaturated fatty acid produced according to the method of claim 16.

20. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 17 in an amount sufficient to effect said prevention or treatment.

21. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO: 2 (Figure 22), wherein said isolated nucleotide sequence encodes a polypeptide having enlongase activity.

22. The isolated nucleotide sequence of claim 21 wherein said sequence is represented by SEQ ID NO: 2.

23. A purified protein encoded by said nucleotide sequence of claims 21 or 22.

24. A purified polypeptide which elongates polyunsatured fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 23.

25. A method of producing an elongase enzyme comprising the steps of:

(a) isolating said nucleotide sequence represented by SEQ ID NO: 2 (Figure 22);

(b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;

(c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

26. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO: 2 (Figure 22) operably linked to b) a promoter.

27. A host cell comprising said vector of claim 26.

28. A plant cell, plant or plant tissue comprising said vector of claim 26, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

29. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 28.

30. A transgenic plant comprising said vector of claim 26, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

31. A transgenic, non-human mammal whose genome comprises a DNA sequence, from M. alpina, encoding an elongase operably linked to a promoter.

32. The transgenic, non-human mammal of claim 31, wherein said DNA sequence is represented by SEQ ID NO:2 (Figure 22).

33. A fluid produced by said transgenic, non-human mammal of claim 32 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

34. A method for producing a polyunsaturated fatty acid comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:2 (Figure 22);

b) constructing a vector comprising said isolated nucleotide sequence;

c) introducing said vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by said isolated nucleotide sequence; and

d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

35. The method according to claim 34 further comprising the step of exposing said expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

36. The method of claim 35 further comprising the step of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

37. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 34, said another polyunsaturated fatty acid produced according to the method of claim 35, and said final

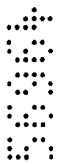
polyunsatured fatty acid produced according to the method of claim 36.

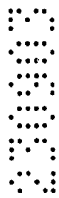
38. A pharmaceutical composition comprising 1) at least one polyunsatured fatty acid selected from the group consisting of said product polyunsatured fatty acid produced according to the method of claim 34, said another polyunsatured fatty acid produced according to the method of claim 35, and said final polyunsatured fatty acid produced, according to the method of claim 36 and 2) a pharmaceutically acceptable carrier.

39. An animal feed comprising at least one polyunsatured fatty acid selected from the group consisting of said product polyunsatured fatty acid produced according to the method of claim 34, said another polyunsatured fatty acid produced according to the method of claim 35 and said final polyunsatured fatty acid produced according to the method of claim 36.

40. A method of preventing or treating a condition caused by insufficient intake of polyunsatured fatty acids comprising administering to said patient said nutritional composition of claim 37 in an amount sufficient to effect said prevention or treatment.

41. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO: 3 (Figure 43), wherein said isolated nucleotide sequence encodes a polypeptide having elongase activity.





42. The isolated nucleotide sequence of claim 41 wherein said sequence is represented by SEQ ID NO:3.

43. A purified protein encoded by said nucleotide sequence of claims 41 or 42.

44. A purified polypeptide which elongates polyunsaturated fatty acids or monounsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 44.

45. A method of producing an elongase enzyme comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:3 (Figure 43);

b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;

c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

46. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:3 (Figure 43) operably linked to b) a promoter.

47. A host cell comprising said vector of claim 46.

48. A plant cell, plant or plant tissue comprising said vector of claim 46, wherein expression of said nucleotide sequence of said vector results in

production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

49. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 48.

50. A transgenic plant comprising said vector of claim 46, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

51. A transgenic, non-human mammal whose genome comprises a human DNA sequence encoding an elongase operably linked to a promoter.

52. The transgenic, non-human mammal of claim 51, wherein said DNA sequence is represented by SEQ ID NO:3 (Figure 43).

53. A fluid produced by said transgenic, non-human mammal of claim 52 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

54. A method for producing a polyunsaturated fatty acid comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:3 (Figure 43);

b) constructing a vector comprising said isolated nucleotide sequence;

c) introducing said vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by said isolated nucleotide sequence; and

d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

55. The method according to claim 54 further comprising the step of exposing said product polyunsaturated fatty acid to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

56. The method of claim 55 further comprising the step of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

57. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 54, said another polyunsaturated fatty acid produced according to the method of claim 55, and said final

polyunsatured fatty acid produced according to the method of claim 56.

58. A pharmaceutical composition comprising 1) at least one polyunsatured fatty acid selected from the group consisting of said product polyunsatured fatty acid produced according to the method of claim 54, said another polyunsatured fatty acid produced according to the method of claim 55, and said final polyunsatured fatty acid produced according to the method of claim 56 and 2) a pharmaceutically acceptable carrier.

59. An animal feed comprising at least one polyunsatured fatty acid selected from the group consisting of said product polyunsatured fatty acid produced according to the method of claim 54, said another polyunsatured fatty acid produced according to the method of claim 55 and said final polyunsatured fatty acid produced according to the method of claim 56.

60. A method of preventing or treating a condition caused by insufficient intake of polyunsatured fatty acids comprising administering to said patient said nutritional composition of claim 59 in an amount sufficient to effect said treatment.

61. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO: 4 (Figure 46), wherein said isolated nucleotide sequence encodes a polypeptide having elongase activity.

62. The isolated nucleotide sequence of claim 61 wherein said sequence is represented by SEQ ID NO:4.

63. A purified protein encoded by said nucleotide sequence of claims 61 or 62.

64. A purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 63.

65. A method of producing an elongase enzyme comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:4 (Figure 46);

b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;

c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

66. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:4 (Figure 46) operably linked to b) a promoter.

67. A host cell comprising said vector of claim 66.

68. A plant cell, plant or plant tissue comprising said vector of claim 66, wherein expression of said nucleotide sequence of said vector results in

production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

69. One or more plant oils or fatty acids expressed by said plant cell, plant or plant tissue of claim 68.

70. A transgenic plant comprising said vector of claim 66, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

71. A transgenic, non-human mammal whose genome comprises a DNA sequence, from C. elegans, encoding an elongase operably linked to a promoter.

72. A fluid produced by said transgenic, non-human mammal of claim 71 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

73. A method for producing a polyunsaturated fatty acid comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:4 (Figure 46);

b) constructing a vector comprising said isolated nucleotide sequence;

c) introducing said vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by said isolated nucleotide sequence; and

d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

74. The method according to claim 73 further comprising the step of exposing said expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

75. The method of claim 74 further comprising the steps of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

76. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 73, said another polyunsaturated fatty acid produced according to the method of claim 74, and said final polyunsaturated fatty acid produced according to the method of claim 75.

77. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty

acid produced according to the method of claim 73, said another polyunsaturated fatty acid produced according to the method of claim 74, and said final polyunsaturated fatty acid produced according to the method of claim 75 and 2) a pharmaceutically acceptable carrier.

78. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 73, said another polyunsaturated fatty acid produced according to the method of claim 74 and said final polyunsaturated fatty acid produced according to the method of claim 75.

79. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 76 in an amount sufficient to effect said prevention or treatment.

80. A nutritional composition as defined in claim 76 and substantially as herein described with reference to the Nutritional Compositions.

81. A method of producing an elongase enzyme, substantially as herein described with reference to Examples II and III or Examples VII – X.

82. The isolated nucleotide sequence of claims 1 or 2 wherein said sequence encodes a functionally active elongase which utilizes a polyunsatured fatty acid as a substrate.

83. The nucleotide sequence of claim 1 wherein said sequence is derived from a fungus of the genus Mortierella.

84. The nucleotide sequence of claim 83 wherein said fungus is of the species *alpina*.

85. The host cell of claim 7, wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

86. The host cell of claim 85 wherein said prokaryotic cell is selected from the group consisting of *E. coli*, Cyanobacteria, and *B. subtilis*.

87. The host cell of claim 85 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

88. The host cell of claim 87 wherein said fungal cell is a yeast cell.

89. The host cell of claim 88 wherein said yeast cell is selected from the group consisting of *Saccharomyces cerevisiae, Saccharomyces carlsbergenis*, Candida spp., *Lipomyces starkey, Yarrowia lipolytica*, Kluyveromyces spp., Hansenula spp. Trichoderma spp. and Pichia spp.

90. The host cell of claim 89 wherein said host cell is *Saccharomyces cerevisiae*.

91. The method of claim 5 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

92. The method of claim 91 wherein said prokaryotic cell is selected from the group consisting of *E. coli*, cyanobacteria, and *B. subtilis*.

93. The method of claim 91 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

94. The method of claim 93 wherein said fungal cell is a yeast cell.

95. The method of claim 94 wherein said yeast cell is selected from the group consisting of *Saccharomyces cerevisiae, Saccharomvces carlsbergenis,* Candida spp., *Lipomyces starkey, Yarrowia lipolytica,* Kluyveromyces spp., Hansenula spp. Trichoderma spp. and Pichia spp.

96. The method of claim 95 wherein said yeast cell is *Saccharomyces cerevisiae*.

97. The sequence of claim 1 wherein the sequence corresponds to or is complementary to at least 60% of the nucleotide sequence shown in SEQ ID NO:1.

98. The sequence of claim 1 wherein the sequence corresponds to or is complementary to at least 70% of the nucleotide sequence shown in SEQ ID NO:1.

99. The sequence of claim 21 wherein the sequence corresponds to or is complementary to at least 45% of the nucleotide sequence shown in SEQ ID NO:2.

100. The sequence of claim 21 wherein the sequence corresponds to or is complementary to at least 55% of the nucleotide sequence shown in SEQ ID NO:2.

101. The sequence of claim 41 wherein the sequence corresponds to or is complementary to at least 45% of the nucleotide sequence shown in SEQ ID NO:3.

102. The sequence of claim 41 wherein the sequence corresponds to or is complementary to at least 55% of the nucleotide sequence shown in SEQ ID NO:3.

103. The sequence of claim 61 wherein the sequence corresponds to or is complementary to at least 45% of the nucleotide sequence shown in SEQ ID NO:4.

104. The sequence of claim 61 wherein the sequence corresponds to or is complementary to at least 55% of the nucleotide sequence shown in SEQ ID NO:4.

Dated 22 September, 2003
Abbott Laboratories

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

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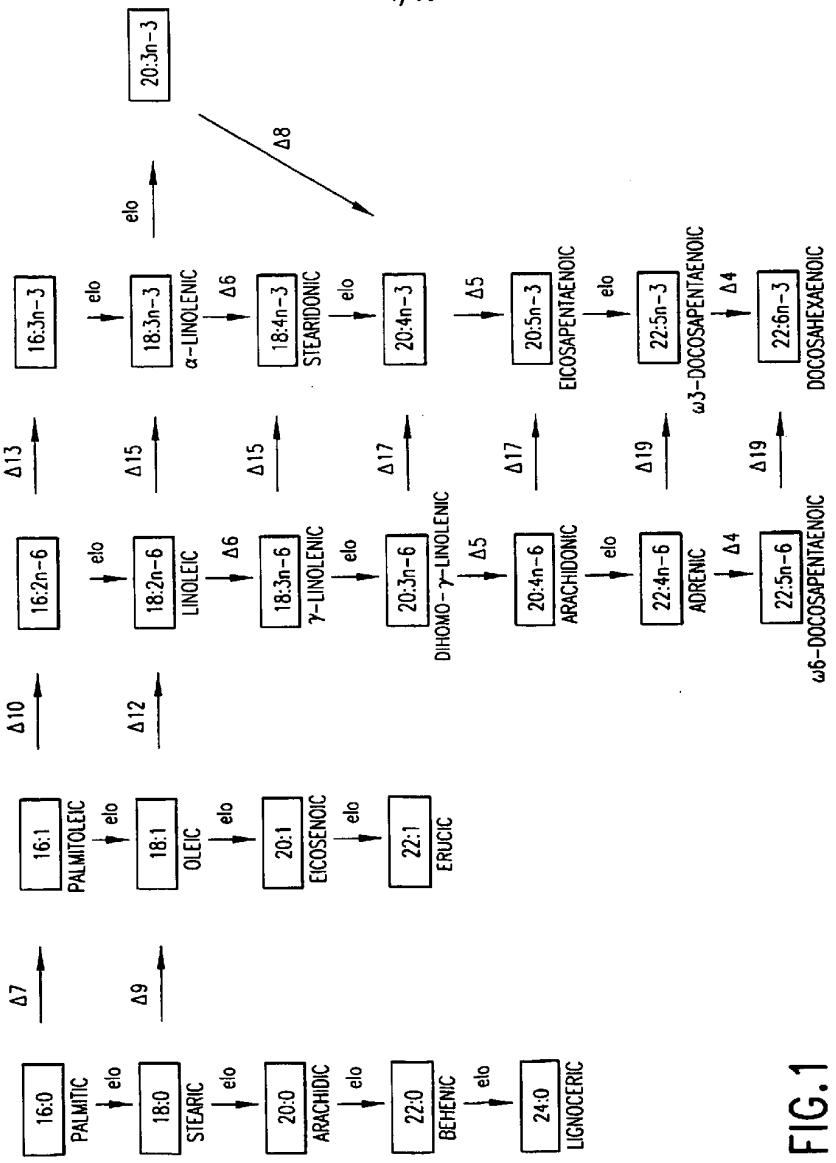

FIG. 1

SUBSTITUTE SHEET (RULE 26)

```
jojobakcs   24 ATLPNFKSSINLHHVKL.GYHYLISNALFLVFIPLLGLASAHLSSFSAHD 72
                 .|||    .|  ::| : |  :|:|.    |  | ||   |    ..
ELO2        66 STLPFVLYAITAYYVIIFGGRFLLSKS..KPF.KLNGLFQLHNLVLTSLS 112

jojobakcs   73 LSLLFDLLLRRNLLPVVVCSFLFVLLATLHFLTRP 106
                  |.||  |:   |.|::|   |: : :  |.|
ELO2       113 LTLLL.LMVEQLVPIIVQHGLYFAICNIGAWTQP 145
```

FIG.2

S	T	L	P	P	V	L	Y	A	I	T	A	Y	Y	V	I	I	F	G	G	R	F	L
TCC	ACC	CTC	CCC	CCC	GTC	CTC	TAC	GCC	ATC	ACC	GCC	TAC	TAC	GTC	ATC	ATC	TTC	GGT	GGT	CGC	TTC	CTC
66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88

<-- RO339

L	S	K	S	K	P	F	K	L	N	G	L	F	Q	L	H	N	L	V	L	T	S	L
CTC	TCC	AAG	TCC	AAG	CCC	TTC	AAG	CTC	AAC	GGT	CTC	TTC	CAG	CTC	CAC	AAC	CTC	GTC	CTC	ACC	TCC	CTC
89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111

S	L	T	L	L	L	L	M	V	E	Q	L	V	P	I	I	V	Q	H	G	L	Y	F
TCC	CTC	ACC	CTC	CTC	CTC	CTC	ATG	GTC	GAG	CAG	CTC	GTC	CCC	ATC	ATC	GTC	CAG	CAC	GGT	CTC	TAC	TTC
112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134

A	I	C	N	I	G	A	W	T	Q	P
GCC	ATC	TGC	AAC	ATC	GGT	GCC	TGG	ACC	CAG	CCC
135	136	137	138	139	140	141	142	143	144	145

FIG.3

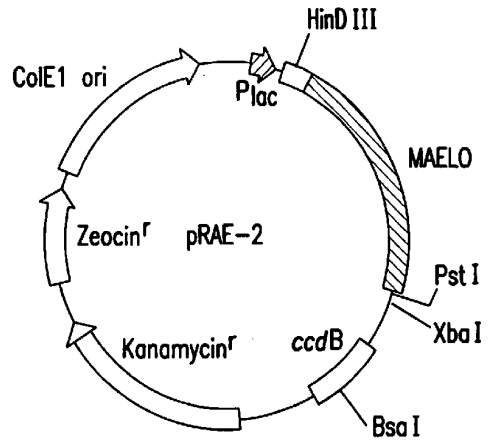
HinD III
ColE1 ori
Plac
MAELO
Zeocin^r
pRAE-2
Pst I
Xba I
Kanamycin^r
ccdB
Bsa I

FIG.4A

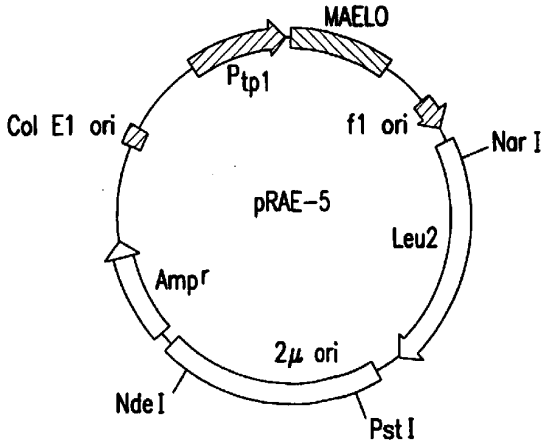
MAELO
Ptp1
Col E1 ori
f1 ori
Nar I
pRAE-5
Leu2
Amp^r
2μ ori
Nde I
Pst I

FIG.4B

pRAE-5 GAATTCAGG * * * * * * * * *CATGGCCGCCGCCGCAATCTTGGACAA

pRAE-6 GAATTCAGGCATCTCATGGATCCGCCATGGCCGCCGCAATCTTGGACAA

EcoRI BamHI NcoI

FIG.5

```
   1 ATGGCCGCCG CAATCTTGGA CAAGGTCAAC TTCGGCATTG ATCAGCCCTT
  51 CGGAATCAAG CTCGACACCT ACTTTGCTCA GGCCTATGAA CTCGTCACCG
 101 GAAAGTCCAT CGACTCCTTC GTCTTCCAGG AGGGCGTCAC GCCTCTCTCG
 151 ACCCAGAGAG AGGTCGCCAT GTGGACTATC ACTTACTTCG TCGTCATCTT
 201 TGGTGGTCGC CAGATCATGA AGAGCCAGGA CGCCTTCAAG CTCAAGCCCC
 251 TCTTCATCCT CCACAACTTC CTCCTGACGA TCGCGTCCGG ATCGCTGTTG
 301 CTCCTGTTCA TCGAGAACCT GGTCCCCATC CTCGCCAGAA ACGGACTTTT
 351 CTACGCCATC TGCGACGACG GTGCCTGGAC CCAGCGCCTC GAGCTCCTCT
 401 ACTACCTCAA CTACCTGGTC AAGTACTGGG AGTTGGCCGA CACCGTCTTT
 451 TTGGTCCTCA AGAAGAAGCC TCTTGAGTTC CTGCACTACT TCCACCACTC
 501 GATGACCATG GTTCTCTGCT TTGTCCAGCT TGGAGGATAC ACTTCAGTGT
 551 CCTGGGTCCC TATTACCCTC AACTTGACTG TCCACGTCTT CATGTACTAC
 601 TACTACATGC GCTCCGCTGC CGGTGTTCGC ATCTGGTGGA AGCAGTACTT
 651 GACCACTCTC CAGATCGTCC AGTTCGTTCT TGACCTCGGA TTCATCTACT
 701 TCTGCGCCTA CACCTACTTC GCCTTCACCT ACTTCCCCTG GGCTCCCAAC
 751 GTCGGCAAGT GCGCCGGTAC CGAGGGTGCT GCTCTCTTTG GCTGCGGACT
 801 CCTCTCCAGC TATCTCTTGC TCTTTATCAA CTTCTACCGC ATTACCTACA
 851 ATGCCAAGGC CAAGGCAGCC AAGGAGCGTG GAAGCAACTT TACCCCCAAG
 901 ACTGTCAAGT CCGGCGATC GCCCAAGAAG CCCTCCAAGA GCAAGCACAT
 951 CTAA
```

FIG.6

```
  1  MAAAILDKVN FGIDQPFGIK LDTYFAQAYE LVTGKSIDSF VFQEGVTPLS

 51  TQREVAMWTI TYFVVIFGGR QIMKSQDAFK LKPLFILHNF LLTIASGSLL

101  LLFTENLVPI LARNGLFYAI CDDGAWTQRL ELLYYLNYLV KYWELADTVF

151  LVLKKKPLEF LHYFHHSMTM VLCFVQLGGY TSVSWVPITL NLTVHVFMYY

201  YYMRSAAGVR IWWKQYLTTL QIVQFVLDLG FTYFCAYTYF AFTYFPWAPN

251  VGKCAGTEGA ALFGCGLLSS YLLLFINFYR ITYNAKAKAA KERGSNFTPK

301  TVKSGGSPKK PSKSKHI*
```

FIG.7

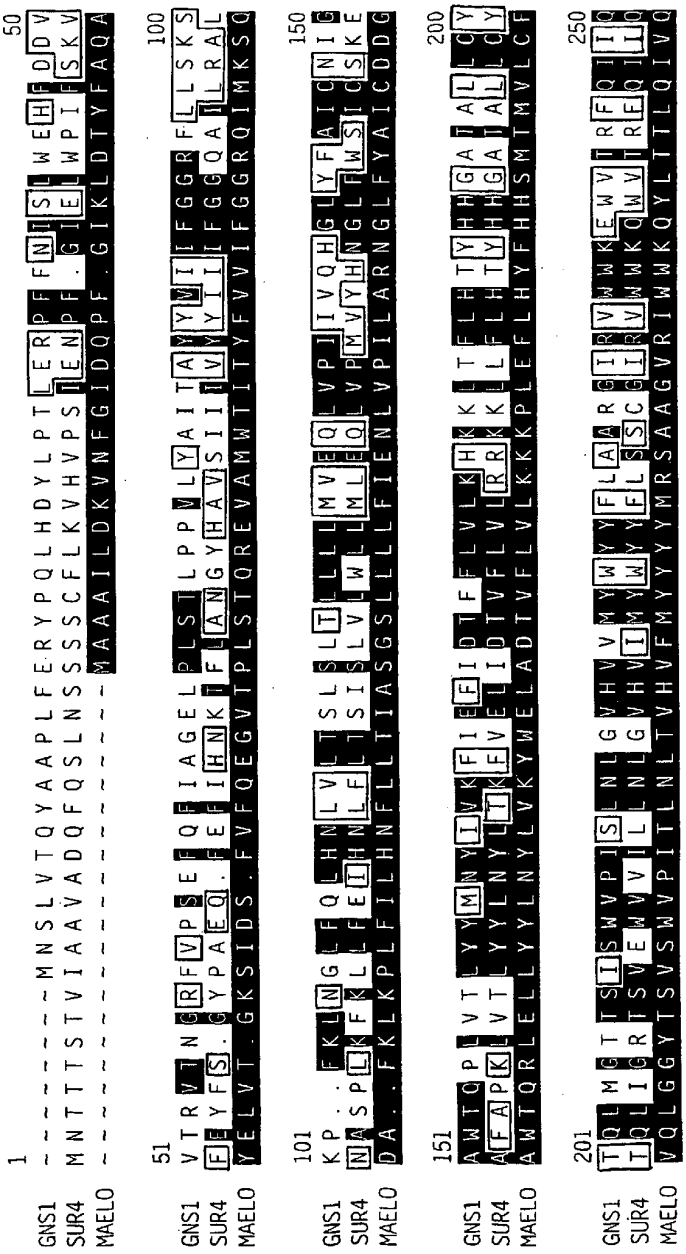

FIG.8A

SUBSTITUTE SHEET (RULE 26)

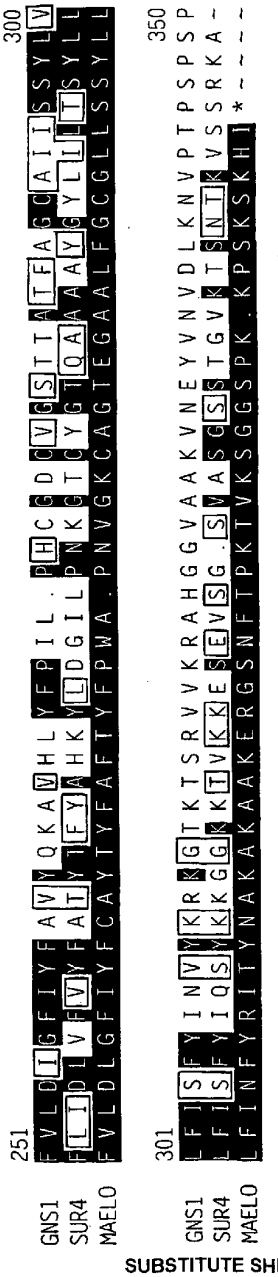
251
300
GNS1
SUR4
MAELO
301
350
GNS1
SUR4
MAELO
351
GNS1
SUR4
MAELO

FIG.8B

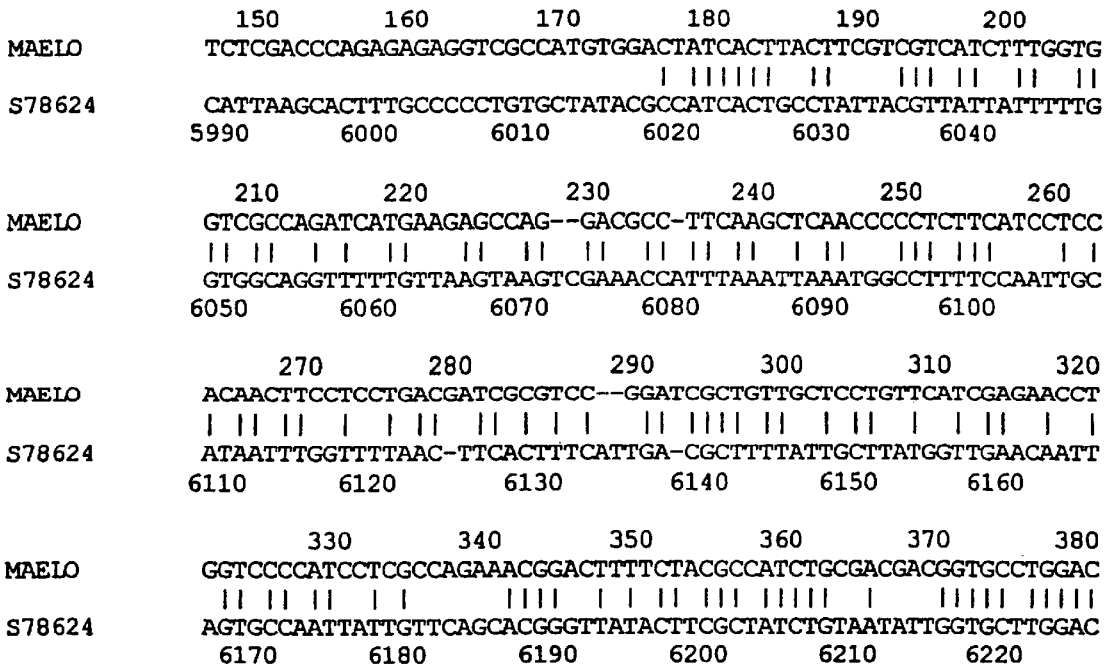

FIG.9A

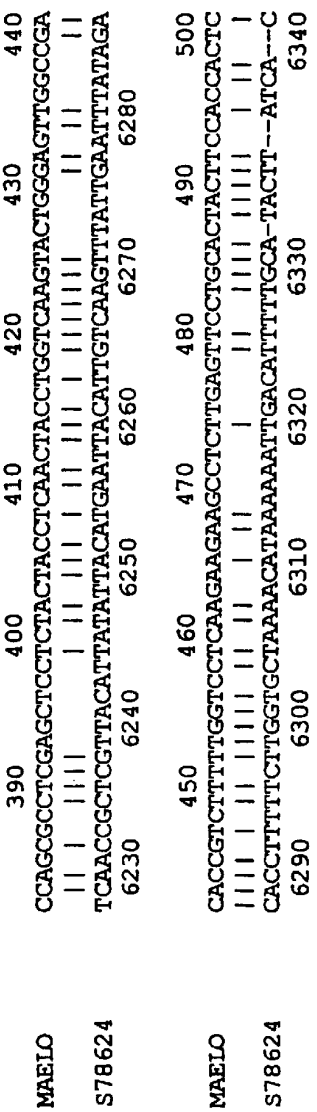
390 400 410 420 430 440
MAEIO CCAGCGCCTCGAGCTCCTCTACTACCTCAACTACCTGGTCAAGTACTGGGAGTTGGCCGA
S78624 TCAACCGCTCGTTACATTATATTACATGAATTACATTGTCAAGTTTATTGAATTTATAGA
6230 6240 6250 6260 6270 6280
450 460 470 480 490 500
MAEIO CACCGTCTTTTTGGTCCTCAAGAAGAAGCCTCTTGAGTTCCTGCACTACTTCCACCACTC
S78624 CACCTTTTTCTTGGTGCTAAAACATAAAAAATTGACATTTTTGCA-TACTT--ATCA--C
6290 6300 6310 6320 6330 6340
510 520 530 540 550
MAEIO GATGACCATGGTTCTCTGCTTTGT----CCAGCTTGGAGGATA-CACTTCAGTGTCCTGG
S78624 CATGGCGCTACTGCCTTATTATGTTACACCCAATTGATGGGCACCACATCTATTTCTTGG
6350 6360 6370 6380 6390 6400
560 570 580 590 600 610
MAEIO GTCCCTATTACCCTCAACTTGACTGTCCACGTCTTCATGTACTACTACATGCGCTCC
S78624 GTCCCTATTTCATTGAACCTTGGTGTTCACGTGGTTATGTATTGGTACTATTT---CTTG
6410 6420 6430 6440 6450

FIG.9B

Host(plasmid)	334(pCGN7875)	334(pYES2)	334(pYX242)	334(pRAE-5)	334(pRAE-6)	334(pYX242)	334(pRAE-5)
Added substrate	25 µM OA	25 µM OA	25 µM GLA	25 µM GLA	25 µM GLA	no substrate	no substrate
Fatty acid	lipid (µg)	lipid (µg)	lipid (µg)	lipid (µg)	lipid (µg)	lipid (µg)	lipid (µg)
C16:0	11.948	23.601	35.123	92.011	85.160	16.294	25.34
C16:1	30.665	71.217	32.789	315.464	115.456	56.183	113.913
C18:0	6.185	9.704	10.515	22.628	18.879	5.535	11.092
C18:1n-9	35.340	57.429	33.989	154.386	106.881	28.388	51.538
C18:3n-6			48.856	58.084	12.434		
C20:0			0.474	0.710	0.244		
C20:1n-9	(0.375%)* 0.352	(0.309%)* 0.527		1.405	0.867		0.516
C20:3n-6	ND	ND	(0.092%)* 0.226	(0.324%)* 2.504	(0.269%)* 1.006	ND	ND
C22:0				0.460			
C22:1n-9				0.321	0.315		
C24:0					1.825		0.999
Total Lipid	93.760	170.490	245.090	771.690	374.420	112.99	256.52
ND = Not Detected							
*% total fatty acid							

FIG.10A

Host(plasmid)	334(pYX242)	334(pYX242)	334(pRAE-5)	334(pRAE-5)	334(pRAE-6)
Added substrate	25 µM GLA	25 µM GLA	25 µM GLA	25 µM GLA	25 µM GLA
Fatty acid	lipid (µg)	lipid (µg)	lipid (µg)	lipid (µg)	lipid (µg)
C16:0	60.683	61.487	100.998	96.193	66.761
C16:1	79.838	79.586	359.754	220.440	87.359
C18:0	9.784	10.106	15.317	15.165	16.744
C18:1n-9	38.536	39.936	108.472	89.637	71.631
C18:3n-6	17.974	17.833	82.866	56.596	17.766
C20:0			0.510	0.570	
C20:1n-9					
C20:3n-6	(0.136%)* 0.389	(0.130%)* 0.374	(0.336%)* 3.035	(0.401%)* 2.689	(0.353%)* 1.185
C22:0			0.414		
C22:1n-9				0.383	
C24:0			1.513	1.626	
Total Lipid	285.560	288.045	902.560	671.113	335.496
*% total fatty acid					

FIG.10B

Host(plasmid)	334(pRAE-5/pCGR4)	334(pYX242/pYES2)		Host(plasmid)	334(pRAE-5/pCGR4)	334(pYX242/pYES2)
Added substrate	25 µM GLA	25 µM GLA		Added substrate	25 µM GLA	25 µM GLA
Fatty Acid	lipid (µg)	lipid (µg)			lipid (µg)	lipid (µg)
C16:0	41.050	37.169		C16:0	96.986	32.221
C16:1	99.393	100.552		C16:1n-7	209.667	62.757
C18:0	34.432	27.852		C18:0	80.418	14.027
C18:1	110.631	92.786		C18:1n-9	207.104	28.701
C18:3n-6	**15.004**	**7.924**		**C18:3n-6**	**25.264**	**10.543**
C20:0	0.643	0.574		C20:0	2.038	
C20:1	1.996	1.684		C20:1n-9	3.591	
C20:3n-6	**0.542**	**0.607**		**C20:3n-6**	**1.284**	**0.326**
C20:4n-6	**0.579**			**C20:4n-6**	**1.392**	
C22:0	1.242	2.604		C22:0	1.124	
C24:0	4.754	4.563		C24:0	3.952	
Total Lipid	334	300		Total Lipid	756	197

FIG.11

Host(plasmid)	334(pYX242)	334(pRAE-5)	334(pRELO-1)	334(pRELO-2)
Added substrate	25 µM GLA	25 µM GLA	25 µM GLA	25 µM GLA
	25°C/48hrs	25°C/48hrs	25°C/48hrs	25°C/48hrs
Fatty acid	lipid (µg)	lipid (µg)	lipid (µg)	lipid (µg)
C16:0	28.7	76.707	84.424	77.445
C16:1	0.729	2.513	1.532	1.056
C18:0	7.432	15.761	27.17	21.32
C18:1n-9	28.9	77.323	109.419	82.844
C18:3n-6	9.729	29.236	19.085	**18.804**
C20:0		0.643	0.522	0.537
C20:1n-9		0.77	0.426	0.299
C20:3n-6	**(0.185%)* 0.374**	**(0.279%)* 1.472**	**(0.153%)* 0.748**	**(0.200%)* 0.832**
C22:0		0.451		
C22:1n-9			0.224	
C24:0		0.918		
Total Lipid	202	527	490	416
*%total fatty acid				

FIG.12

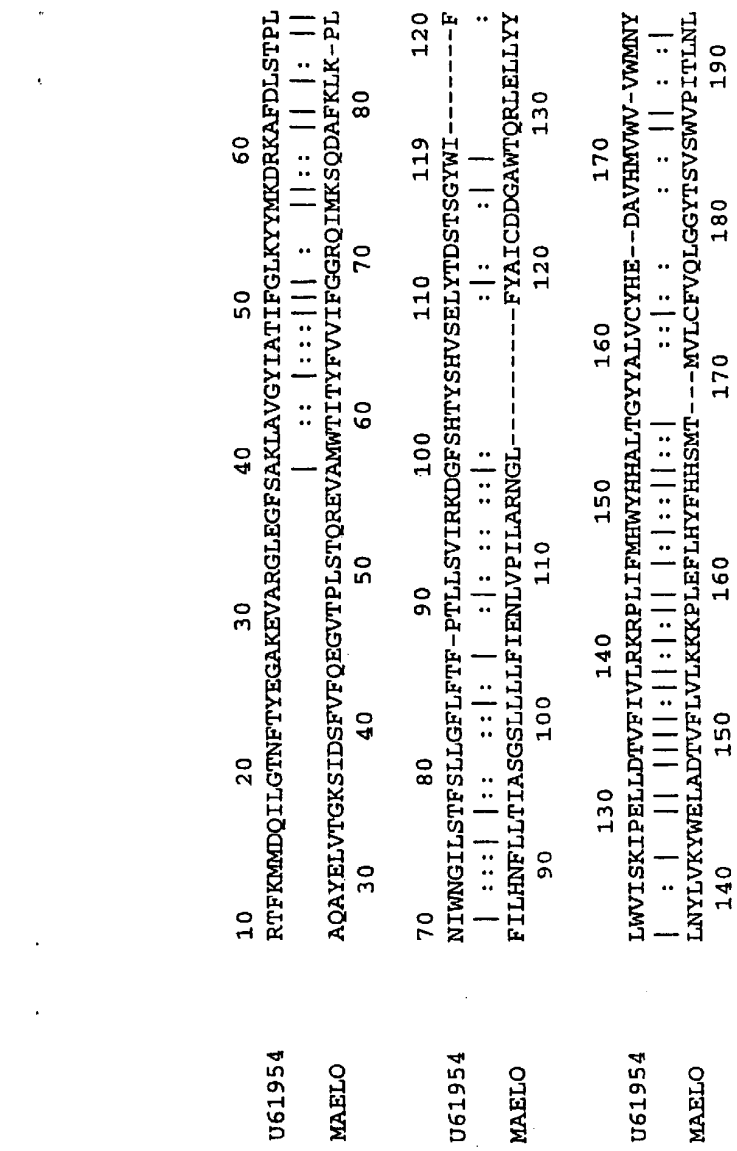
10 20 30 40 50 60
U61954 RTFKMMDQILGTNFTYEGAKEVARGLEGFSAKLAVGYIATIFGLKYYMKDRKAFDLSTPL
MAELO AQAYELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLK-PL
30 40 50 60 70 80
70 80 90 100 110 119 120
U61954 NIWNGILSTFSLLGFLFTF-PTLLSVIRKDGFSHTYSHVSELYTDSTSGYWI-------F
MAELO FILHNFLLTIASGSLLLLFIENLVPILARNGL----------FYAICDDGAWTQRLELLYY
90 100 110 120 130
130 140 150 160 170
U61954 LWVISKIPELLDTVFIVLRKRPLIFMHWYHHALTGYYALVCYHE--DAVHMVWV-VWMNY
MAELO LNYLVKYWELADTVFLVLKKKPLEFLHYFHHSMT---MVLCFVQLGGYTSVSWVPITLNL
140 150 160 170 180 190

FIG. 13A

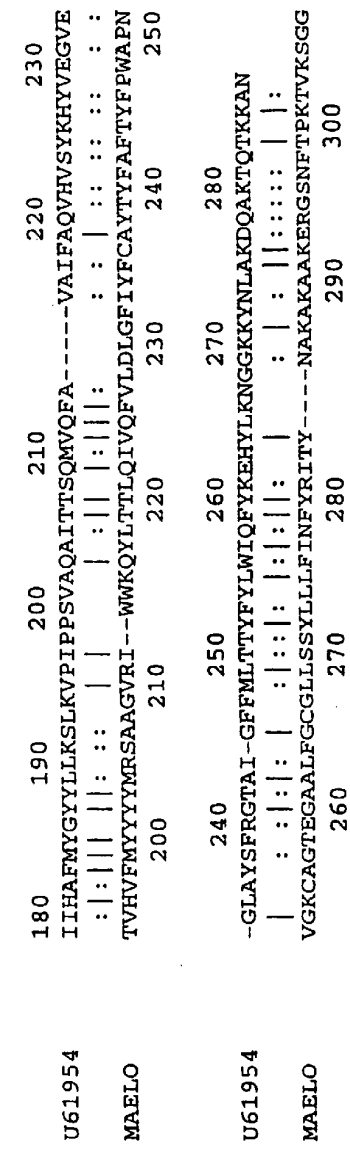
180 190 200 210 220 230
U61954 IIHAFMYGYYLLKSLKVPIPPSVAQAITTSQMVQFA-----VAIFAQVHVSYKHYVEGVE
MAELO TVHVFMYYYYMRSAAGVRI--WWKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPN
200 210 220 230 240 250
240 250 260 270 280
U61954 -GLAYSFRGTAI-GFFMLTTYFYLWIQFYKEHYLKNGGKKYNLAKDQAKTQTKKAN
MAELO VGKCAGTEGAALFGCGLLSSYLLLLFINFYRITY----NAKAKAAKERGSNFTPKTVKSGG
260 270 280 290 300
MAELO SPKKPSKSKHIX
310

FIG. 13B

```
                50        60        70        80        90       100
Z68749  SLLTNQDEVFPHIRARRFIQEHFGLFVQMAIAYVILVFSIKRFMRDREPFQLTTALRLWN
                                    |:| :::|: :::|:::: |:|   : | |
MAELO   ELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFILHN
               30        40        50        60        70        80

                110       120       130       140       150       160
Z68749  FFLSVFSIYGSWTMFPF--MVQQIRLYGLYGCGCEALSNLPSQAEYWLFLTILSKAVEFV
        |:|:: |  ||  :: : :| :  ||:  |: :  :: |  :|: | |  |::
MAELO   FLLTIAS--GSLLLLFIENLVPILARNGLFYAICDD-GAWTQRLELLYYLNYILVKYWELA
               90        100       110       120       130       140

                170       180       190       200       210       220
Z68749  DTFFLVLRKKPLIFLHWYHHMATFVFFCSNYPTPSSQSRVGVIVNLFVHAFMYPYYFTRS
        || |||||:|||| |||::|| |:|:  :    :| | | : :|| ||:|||| ||:  :
MAELO   DTVFLVLKKKPLEFLHYFHHSMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYMRSA
               150       160       170       180       190       200
```

FIG. 14A

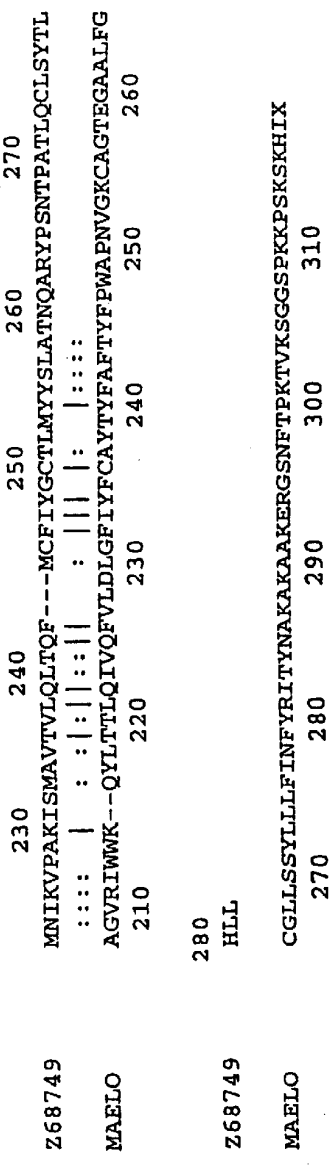
230 240 250 260 270
Z68749 MNIKVPAKISMAVTVLQLTQF---MCFIYGCTLMYYSLATNQARYPSNTPATLQCLSYTL
:::: | : :|:||::|| : ||| |: |::::
MAELO AGVRIWWK--QYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVGKCAGTEGAALFG
210 220 230 240 250 260
280
Z68749 HLL
270 280 290 300 310
MAELO CGLLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSKHIX

FIG. 14B

```
                10        20        30        40
AF003134        MLYSITRRCYTFFVTSLHFYQLYVTECLENVIFNVLVNGQSINSRWKD
                          |:|: :   |:| : : :::: :|:  |:   :::: :
MAELO           MAAAILDKVNFGIDQPFGIKLDTYFAQA---YELVTGKSIDSFVFQEGVT--PLSTQREV
                    10        20        30        40        50

                50        60        70        80        90        100
AF003134        AEKTITSFPFHF-------PQTFFQQPHILTLHFLFFVFVSVTLVTVFKKPKCEFPHSLA
                |  |||  |   |       |  |:   :: ||  :::::: |  :|: :|
MAELO           AMWTITYFVVIFGGRQIMKSQDAFKLKPLFILHNFLLTIASGSLLLLFIENLVPILARNG
                    60        70        80        90        100       110
```

FIG.15

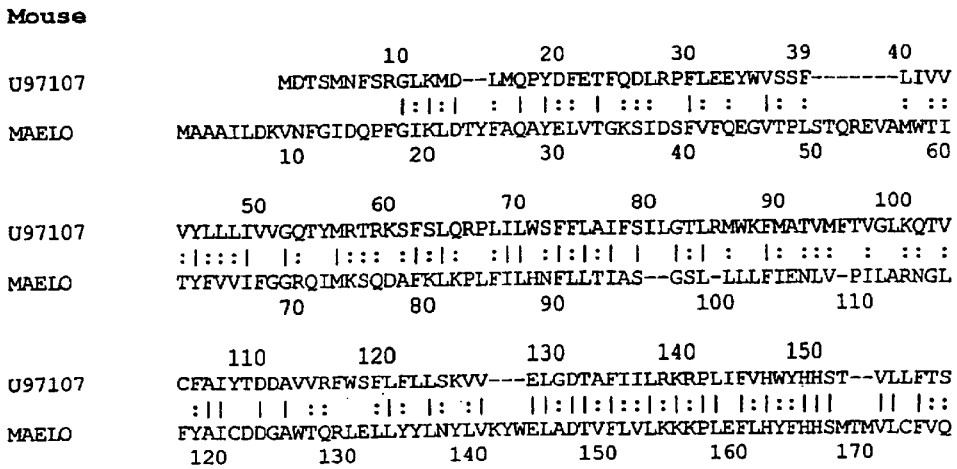
Mouse
U97107
MAELO
MDTSMNFSRGLKMD--LMQPYDFETFQDLRPFLEEYWVSSF-------LIVV
MAAAILDKVNFGIDQPFGIKLDTYFAQAYELVTGKSIDSFVFQEGVTPLSTQREVAMWTI
VYLLLIVVGQTYMRTRKSFSLQRPLILWSFFLAIFSILGTLRMWKFMATVMFTVGLKQTV
TYFVVIFGGRQIMKSQDAFKLKPLFILHNFLLTIAS--GSL-LLLFIENLV-PILARNGL
CFAIYTDDAVVRFWSFLFLLSKVV---ELGDTAFIILRKRPLIFVHWYHHST--VLLFTS
FYAICDDGAWTQRLELLYYLNYLVKYWELADTVFLVLKKKPLEFLHYFHHSMTMVLCFVQ

FIG. 16A

```
Mouse

                160       170       180       190       200       210
                EGYKNKVPSGGWF-MTMNFGVHSVMYTYYTMKQAAKLKHPNLLPMVITSLQILQWLG---
                :|  ::|   :|  :|:|: ||  || ||  :|| ::      : :|:|||:|:||
U97107          LGGYTSV---SWVPTTLNLTVHVFMYTYYTMRSAAGVR--IWWKQYLTTLQIVQFVLDLGF
                   180       190       200       210       220       230

                          220       230       240       250       260
                ------TIFGILNYIWRQEKG-CHTTTEHFFWSFMLYGTYFILFAHFFHRAYLRPKGKVA
                      | |::  : |  : | | |     :::  | ::|::|| :|:: :|   |:|:|
MAELO           IYFCAYTYFAFTYFFWAFNVGKCAGTEGAALFGCGLLSSYLLLFTNFYRITY-NAKAKAA
                      240       250       260       270       280       290

                270
U97107          SKSQX
                ::
MAELO           KERGSNFTPKTVKSGGSPKKPSKSKHIX
                      300       310
```

FIG. 16B

```
Human

                110       120       130       140       150       160
MAELO           NLVPILARNGLFYAICDDGAWTQRLELLYYINYLVKYWELADTVFLVLKKKCPLEFLHYFH
                                          |:||:          :||:|::|:|: | |||::|
AC004050                                  SLLVVKDLITYLLPLCLPGDTIFIILRKQKLIFLHWYH
                                                  10        20        30

                170       180       190       200       210       220
MAELO           HSMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYYMRSAAGVRIWWK--QYLTTLQIV
                |  :::  : :  ::: :   :|:|  ||: || ||   ||| |:  |   :::|  ||:
AC004050        HITVLLYSWYSYKDMVAGGGWFMTMNYGVHAVMYSYYALRAAGFRVSRKFAMFITLSQIT
                  40        50        60        70        80        90

                  230       240       250       260       270       280
MAELO           QFVLDLGFIYFCAYTYFAFTYFPWAPNVGKCAGTEGAALFGCGLLSSYILLFINFYRITY
                |:::       |: :|:::|   |  :  :|  :   :::  :  |||:|| :|:  :|
AC004050        QMIMG------CVVNYLVFC---WMQH-DQCHSHFQNIFWSSIMYLSYIVLFCHFFFEAY
                100               110       120       130       140
```

FIG. 16C

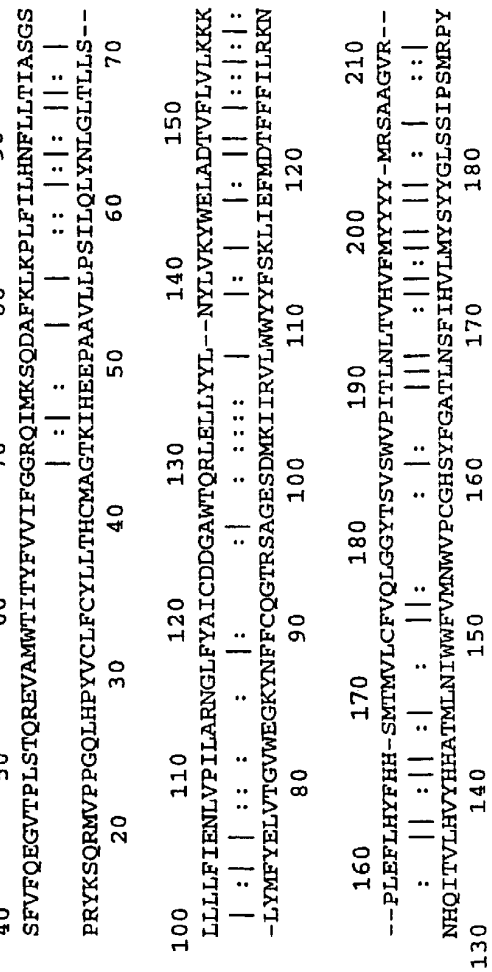
40 50 60 70 80 90
MAELO SFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFILHNFLLTIASGS
I05465 PRYKSQRMVPPGQLHPYVCLFCYLLTHCMAGTKIHEEPAAVLLPSILQLYNLGLTLLS--
20 30 40 50 60 70
100 110 120 130 140 150
MAELO LLLLFIENLVPILARNGLFYAICDDGAWTQRLELLYYL--NYLVKYWELADTVFLVLKKK
I05465 -LYMFYELVTGVWEGKYNFFCQGTRSAGESDMKIIRVLWWYYFSKLIEFMDTFFFILRKN
80 90 100 110 120
160 170 180 190 200 210
MAELO --PLEFLHYFHH-SMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYY-MRSAAGVR--
I05465 NHQITVLHVYHHATMLNIWWFVMNWVPCGHSYFGATLNSFIHVLMYSYYGLSSIPSMRPY
130 140 150 160 170 180

FIG. 17A

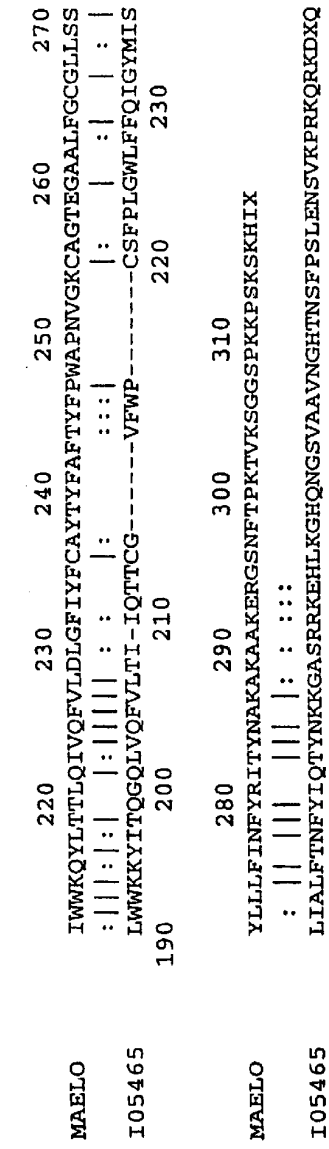
220 230 240 250 260 270
MAELO IWWKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVGKCAGTEGAALFGCGLLSS
I05465 LWWKKYITQGQLVQFVLTI-IQTTCG------VFWP--------CSFPLGWLFFQIGYMIS
190 200 210 220 230
MAELO YLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSKHIX
I05465 LIALFTNFYIQTYNKKGASRRKEHLKGHQNGSVAAVNGHTNSFPSLENSVKPRKQRKDXQ
240 250 260 270 280 290

FIG. 17B

```
  1 MGTDQGKTFT WEELAAHNTK DDLLLAIRGR VYDVTKFLSR HPGGVDTLLL
 51 GAGRDVTPVF EMYHAFGAAD AIMKKYYVGT LVSNELPIFP EPTVFHKTIK
101 TRVEGYFTDR NIDPKNRPEI WGRYALIFGS LIASYYAQLF VPFVVERTWL
151 QVVFAIIMGF ACAQVGLNPL HDASHFSVTH NPTVWKILGA THDFFNGASY
201 LVWMYQHMLG HHPYTNIAGA DPDVSTSEPD VRRIKPNQKW FVNHINQHMF
251 VPFLYGLLAF KVRIQDINIL YFVKTNDAIR VNPISTWHTV MFWGGKAFFV
301 WYRLIVPLQY LPLGKVLLLF TVADMVSSYW LALTFQANHV VEEVQWPLPD
351 ENGIIQKDWA AMQVETTQDY AHDSHLWTSI TGSLNYQAVH HLFPNVSQHH
401 YPDILAIIKN TCSEYKVPYL VKDTFWQAFA SHLEHLRVLG LRPKEE*
```

FIG.18

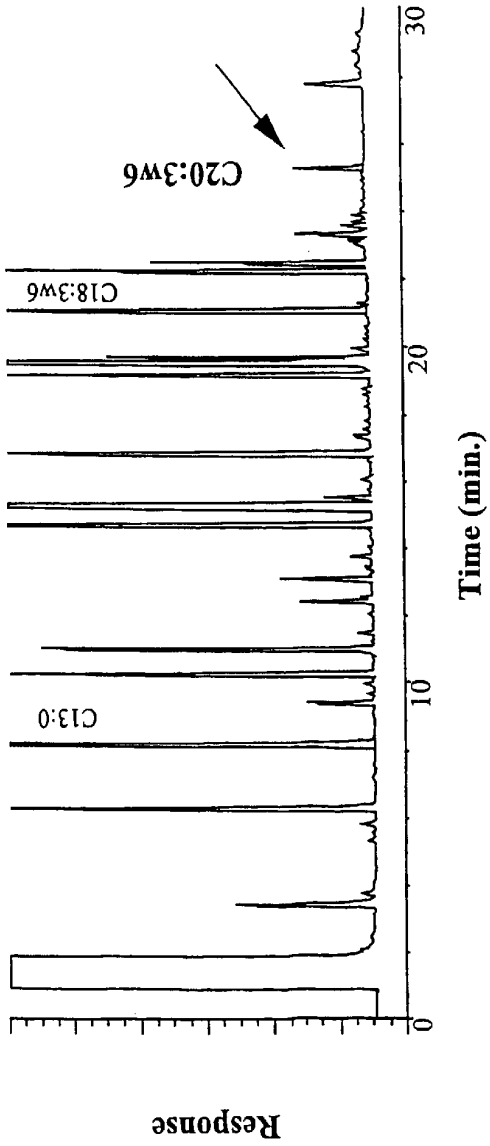

FIG.19

Host(plasmid)	334(MAD708-2)	334 (MAD708-10)	334(MAD708-18)	334 (MAD708-19)	334(MAD708-30)	334 (pRAE5)
Added substrate	25μM GLA	25μM GLA	25μM GLA	25μM GLA	25μM GLA	25μM GLA
			% total lipid			
Fatty Acid						
C16:0	14.1	14.68	14.38	15.45	14.13	13.59
C16:1	42.84	43.42	42.57	38.03	43.58	43.98
C18:0	3.19	3.28	3.63	4.08	3.37	2.04
C18:1n-9	17.66	19.39	19.6	20.8	20.06	10.88
C18:3n-6	**6.65**	**5.58**	**10.24**	**9.46**	**3.56**	**11.14**
C20:0	0.26	0.3	0.32	0.4	0.46	0.57
C20:3n-6	**(47.5%) 6.03**	**(41.2%) 3.92**	**(8.0%) 0.91**	**(21.5%) 2.59**	**(49%) 3.43**	**(3.4%) 0.24**
Total Lipid (μg)	238.47	307.86	188.51	167.31	207.47	466.65

(% conversion) = product/(substrate+product)

FIG.20

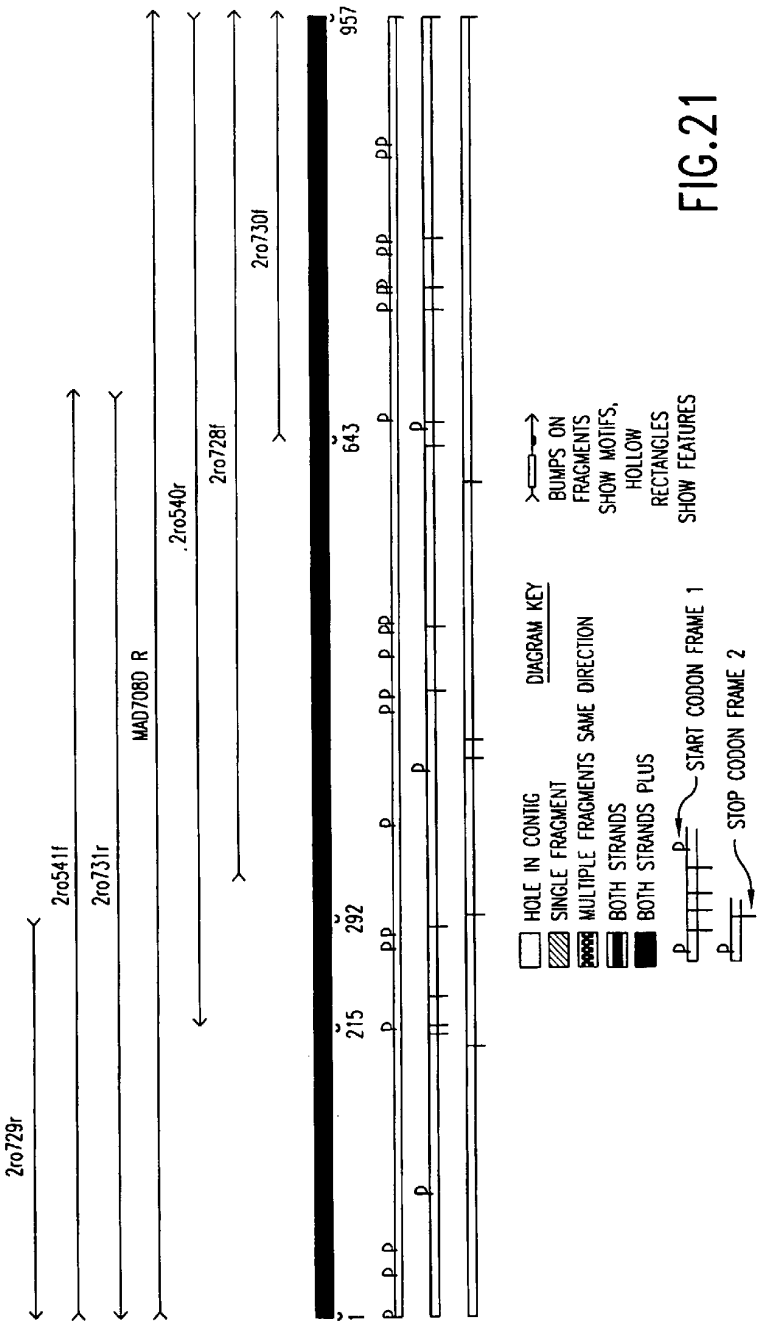
2ro729r
2ro541f
2ro731r
MAD708D R
2ro540r
2ro728f
2ro730f
1
215
292
643
957
DIAGRAM KEY
HOLE IN CONTIG
SINGLE FRAGMENT
MULTIPLE FRAGMENTS SAME DIRECTION
BOTH STRANDS
BOTH STRANDS PLUS
START CODON FRAME 1
STOP CODON FRAME 2
BUMPS ON FRAGMENTS SHOW MOTIFS, HOLLOW RECTANGLES SHOW FEATURES

FIG.21

```
   1 ATGGAGTCGA TTGCGCCATT CCTCCCATCA AAGATGCCGC AAGATCTGTT
  51 TATGGACCTT GCCACCGCTA TCGGTGTCCG GGCCGCGCCC TATGTCGATC
 101 CTCTCGAGGC CGCGCTGGTG GCCCAGGCCG AGAAGTACAT CCCCACGATT
 151 GTCCATCACA CGCGTGGGTT CCTGGTCGCG GTGGAGTCGC CTTTGGCCCG
 201 TGAGCTGCCG TTGATGAACC CGTTCCACGT GCTGTTGATC GTGCTCGCTT
 251 ATTTGGTCAC GGTCTTTGTG GGCATGCAGA TCATGAAGAA CTTTGAGCGG
 301 TTCGAGGTCA AGACGTTTTC GCTCCTGCAC AACTTTTGTC TGGTCTCGAT
 351 CAGCGCCTAC ATGTGCGGTG GGATCCTGTA CGAGGCTTAT CAGGCCAACT
 401 ATGGACTGTT TGAGAACGCT GCTGATCATA CCTTCAAGGG TCTTCCTATG
 451 GCCAAGATGA TCTGGCTCTT CTACTTCTCC AAGATCATGG AGTTTGTCGA
 501 CACCATGATC ATGGTCCTCA AGAAGAACAA CCGCCAGATC TCCTTCTTGC
 551 ACGTTTACCA CCACAGCTCC ATCTTCACCA TCTGGTGGTT GGTCACCTTT
 601 GTTGCACCCA ACGGTGAAGC CTACTTCTCT GCTGCGTTGA ACTCGTTCAT
 651 CCATGTGATC ATGTACGGCT ACTACTTCTT GTCGGCCTTG GGCTTCAAGC
 701 AGGTGTCGTT CATCAAGTTC TACATCACGC GCTCGCAGAT GACACAGTTC
 751 TGCATGATGT CGGTCCAGTC TTCCTGGGAC ATGTACGCCA TGAAGGTCCT
 801 TGGCCGCCCC GGATACCCCT TCTTCATCAC GGCTCTGCTT TGGTTCTACA
 851 TGTGGACCAT GCTCGGTCTC TTCTACAACT TTTACAGAAA GAACGCCAAG
 901 TTGGCCAAGC AGGCCAAGGC CGACGCTGCC AAGGAGAAGG CAAGGAAGTT
 951 GCAGTAA
```

FIG.22

```
  1  MESIAPFLPS KMPQDLFMDL ATAIGVRAAP YVDPLEAALV AQAEKYIPTI
 51  VHHTRGFLVA VESPLARELP LMNPFHVLLI VLAYLVTVFV GMQIMKNFER
101  FEVKTFSLLH NFCLVSISAY MCGGILYEAY QANYGLFENA ADHTFKGLPM
151  AKMIWLFYFS KIMEFVDTMI MVLKKNNRQI SFLHVYHHSS IFTIWWLVTF
201  VAPNGEAYFS AALNSFIHVI MYGYYFLSAL GFKQVSFIKF YITRSQMTQF
251  CMMSVQSSWD MYAMKVLGRP GYPFFITALL WFYMWTMLGL FYNFYRKNAK
301  LAKQAKADAA KEKARKLQ*
```

FIG.23

Host(plasmid)	334(pRPB2)		334 (pYES2)	
Added substrate	25μM GLA		25μM GLA	
	(n=4)			
Fatty Acid	% total lipid			
C16:0	15.65		15.23	
C16:1	35.2		38.59	
C18:0	5.68		5.55	
C18:1n-9	25.55		25.27	
C18:3n-6	**3.1**		**6.75**	
C20:0	0.36		0.14	
C20:3n-6	**(62.0%)**	**5.06**	**(2.6%)**	**0.18**
Total Lipid (μg)	314		247	

(% conversion) = product/(substrate+product)

FIG.24

Host(plasmid)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)
Added substrate	25μM SA	25μM OA	25μM LA	25μM DGLA	25μM AA	25μM Adrenic
	C18:0	C18:1n-9	C18:2n-6	C20:3n-6	C20:4n-6	C22:4n-6
	% total lipid					
Fatty Acid						
C16:0	15.07	14.52	15.74	15.69	16.06	15.15
C16:1	33.7	32.37	32.23	25.65	33.65	33.39
C18:0	*9.78	5.83	5.61	8.33	4.52	5.35
C18:1n-9	31.2	*37.25	26.05	20.15	24.54	28.54
C18:2n-6			*10.4			
C18:3n-6						
C20:2n-6			0.29			
C20:3n-6				*16.5		
C20:4n-6				0.27	*11.7	
C22:4n-6						*7.46
Total Lipid (μg)	132	130	171	55	225	163

*indicates substrate added
(% conversion) = product/(substrate+product)

FIG.25A

Host(plasmid)	334(pRPB2)	334(pRPB2)	334(pRPB2)
Added substrate	25μM ALA	25μM STA	25μM EPA
	C18:3n-3	C18:4n-3	C20:5n-3
	% total lipid		
Fatty Acid			
C16:0	17.32	16.01	20.67
C16:1	27.68	34.31	50.7
C18:0	6.75	5.39	6.14
C18:1n-9	28.4	28.54	
C18:3n-3	*8.39		
C18:4n-3		*1.95	
C20:4n-3		(73.2%) 5.33	
C20:5n-3			*10.33
C22:5n-3			0.25
Total Lipid (μg)	114	199	201

*indicates substrate added
(% conversion) = product/(substrate+product)

FIG.25B

Host(plasmid)	334(pRPB2+PRPE31)	334(pYES2+pYX242)
Added substrate	25μM GLA	25μM GLA
Fatty Acid	% total lipid	
C16:0	15.54	18.26
C16:1	30.16	33.51
C18:0	8.76	5.58
C18:1n-9	27	27.37
C18:3n-6	*2.6	*5.6
C20:0	0.4	0.32
C20:3n-6	**(57.4%) 3.55**	**(2.9%) 0.17**
C20:4n-6	**(27.6%) 1.32**	**ND**
Total Lipid (μg)	254	258

* indicates substrate added
(% conversion) = product/(substrate+product)

FIG. 26A

Host(plasmid)	334(pRPB2+PRPE31)	334(pYES2+pYX242)
Added substrate	25μM STA	25μM STA
Fatty Acid	% total lipid	
C16:0	18	16.4
C16:1	28.37	34.78
C18:0	7.42	5.71
C18:1n-9	26.44	30.15
C18:4n-3	*2.93	*4.57
C20:0	0.25	0.17
C20:4n-3	4.13	0.32
C20:5n-3	**(39%) 1.87**	**(2.1%) .10**
Total Lipid (μg)	257	304

* indicates substrate added
(% conversion) = product/(substrate+product)

FIG. 26B

```
                40        50        60        70        80        90        99
GLELO           VAQAEKYIPTIVHHTRGFLVAVESPLARELPLMNPFHVLLIVLAYLVTVFVGMQIMKNFE
                                                  || :  :| : :::|:|::| | ||||: :
MAELO           GIKLDTYFAQAYELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQD
                  20        30        40        50        60        70

               100       110       120       130       140       150
GLELO           RFEVKTFSLLHNFCLVSISAYMCGGILYE--AYQANYGLFENAADHTFKGLPMAKMIWLF
                 |::| : :|||| |:  |: :   :: :    |  |||     |      :  : :|
MAELO           AFKLKPLFILHNFLLTIASGSLLLLFIENLVPILARNGLFYAICDDGAWTQRLELLYYLN
                  80        90       100       110       120       130

               160       170       180       190       200       210
GLELO           YFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAALNSFI
                |: |  |::||:::|||| : : ||| :|| : :  :| : : :: ::  :||  :
MAELO           YLVKYWELADTVFLVLKK--KPLEFLHYFHHS-MTMVLCFVQLGGYTSVSWVPITLNLTV
                140       150       160       170       180       190
```

FIG. 27A

```
          220       230       240       250                   260
GLELO     HVIMYGYYFLSALGFKQVSFIKFYITRSQMTQF---------CMMSVQS----SWDMYAM
          ||:|| ||: || | :   : | |:| |::||          | ::  :     |  :
MAELO     HVFMYYYYMRSAAGVRI--WWKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVG
             200       210       220       230       240       250

             270       280       290       300       310
GLELO     KVLGRPGYPFFITALLWFYMWTMLGLFYNFYRKNAKLAKQAKADAAKEKARKLQ
          |  |  |  :|  :||  |:      || ||||    :: :||| ||||::
MAELO     KCAGTEGAALFGCGLLSSYLL----LFINFYR----ITYNAKAKAAKERGSNFTPKTVKS
               260       270           280           290       300

MAELO     GGSPKKPSKSKHIX
             310
```

FIG. 27B

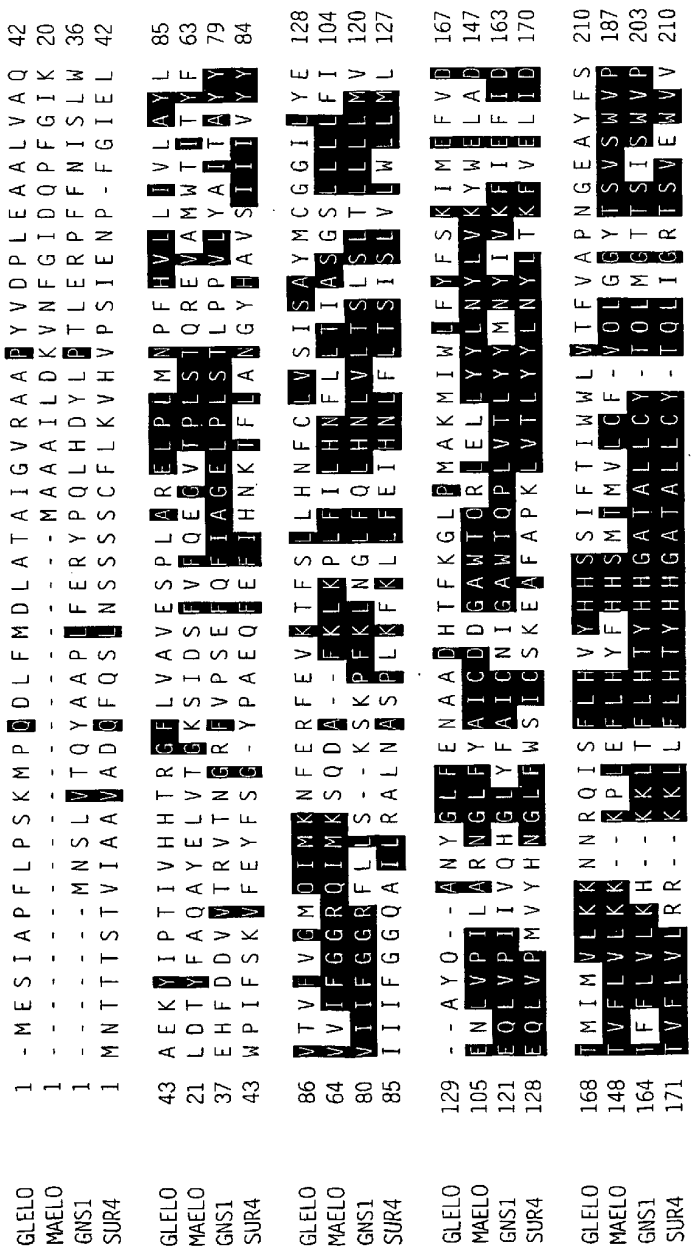

FIG.28A

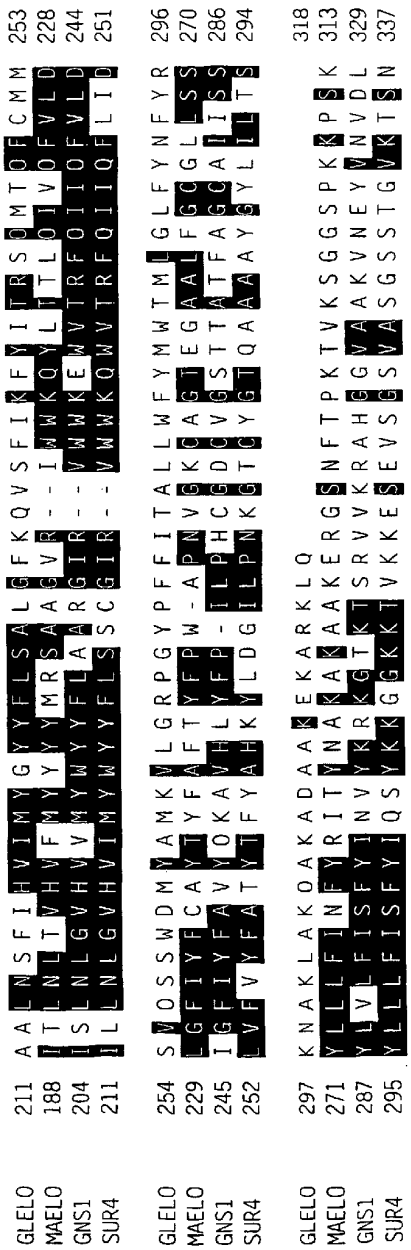
GLELO 211 A L N S F I H V I M Y G Y Y F L S A L G F K Q V S F I K F N I T R S O M T O F C M M 253
MAELO 188 I T L N L T V H V F M Y Y Y M R S A A G V R - - I W W K O Y L I T L O I V O F V L D 228
GNS1 204 I S L N L G V H V V M Y W Y Y F L A A R G I R - - V W W K E W V T R F O I I O F V L D 244
SUR4 211 I L L N L G V H V I M Y W Y Y F L S C G I R - - V W W K O W V T R F O I I O F L I D 251
GLELO 254 S M O S S W D M Y A M K V L G R P G Y P F F I T A L L W F Y M W T M L G L F Y N F Y R 296
MAELO 229 L G F I Y F C A Y T Y F A F T Y F P W - A P N V G K C A G I E G A A L F G C G L L S S 270
GNS1 245 I G F I Y F A V V O K A V H L Y F P - I L P H C G D C V G S T T A T F A G C A I I S S 286
SUR4 252 L V F V Y F A T V T F Y A H K Y L D G I L P N K G T C Y G T Q A A A Y G Y L L T S 294
GLELO 297 K N A K L A K O A K A D A A K E K A R K L Q S N F T P K T V K S G G S P K K P S K 318
MAELO 271 Y L L L F I N F Y R I T Y N A K A K A K E R G S N F T P K T V K K R A H G G V A A K V N E Y W N V D L 313
GNS1 287 Y L V L F I S F Y I N V Y K R K G T K T S R V V K K E S E V S G S V A S G S S T G V K T S N 329
SUR4 295 Y L L L F I S F Y I Q S Y K K G G K K T V K K E S E V S G S V A S G S S T G V K T S N 337
MAELO 314 S K H I
GNS1 330 K N V P T P S P S P K P Q H R R K R
SUR4 338 T K V S S R K A

FIG.28B

```
                30        40        50        60        70        80
MAELO           YELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFILH
                                          :: |-:::: | : |:::: |: : :::::
HS1             STYFKALLGPRDTRVKGWFLLDNYIPTFICSVIYLLIVWLGPKYMRNKQPFSCRGILVVY
                10        20        30        40        50        60

                90       100       110       120       130       140
MAELO           NFLLTIASGSLLLLFIENLVPILARNGLFYAICDDGAWTQRLELLYYL--NYLVKYWELA
                |: ||: |   | :| | :: :  : |:      |  : ::::  |  |: | |:
HS1             NLGLTLLS---LYMFCELVTGVWEGKYNFFCQGTRTAGESDMKIIRVLWWTYYFSKLIEFM
                70        80        90        100       110       120

                150       160       170       180       190       200
MAELO           DTVFLVLKK--KPLEFLHYFHH-SMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYY-
                || |::|:|  : :  || :|| || :  ||:     : |:  |||  :||:|| ||
HS1             DTFFFILRKNNHQITVLHVYHHASMLNIWWFVMNWVPCGHSYFGATLNSFIHVLMYSYYG
                130       140       150       160       170       180
```

FIG. 29A

```
                210       220       230       240       250       260
MAELO   MRSAAGVR--IWWKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVGKCAGTEGA
        : |: ::|  :|||:|:|  |::|||| : :   |:          | |    |:   |
HS1     LSSVPSMRPYLWWKKYITQGQLLQFVLTI-IQTSCGVI---------W-P----CTFPLGW
          190       200       210       220       230

                270       280       290       300       310
MAELO   ALFGCGLLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSKHI
          | | : | : || |||  ||| |: : ::
HS1     LYFQIGYMISLIALFTNFYIQTYNKKGASRRKDHLKDHQNGSMAAVNGHTNSFSPLENNV
            240       250       260       270       280       290

HS1     KPRKLRKDX
            300
```

FIG. 29B

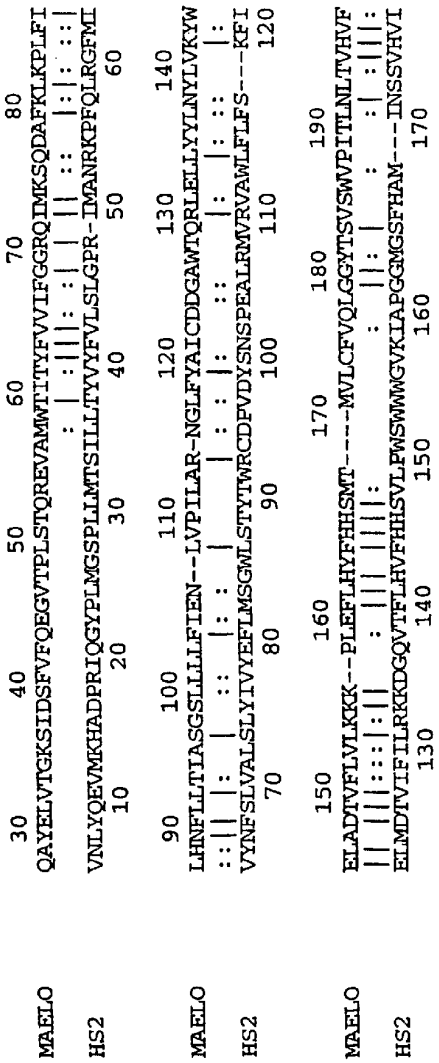

FIG. 30A

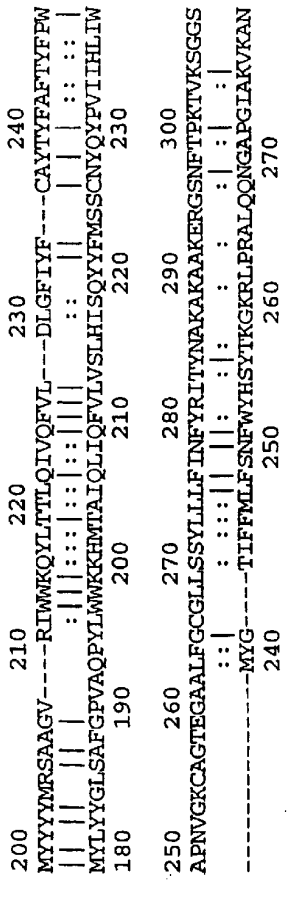
200 210 220 230 240
MAELO MYYYYMRSAAGV----RIWWKQYLTTLQIVQFVL---DLGFIYF---CAYTYFAFTYFPW
HS2 MYLYYGLSAFGFVAQPYLWWKKHMTAIQLIQFVLVSLHISQYYFMSSCNYQYPVIIHLIW
180 190 200 210 220 230
250 260 270 280 290 300
MAELO APNVGKCAGTEGAALFGCGLLSSYLLLFINFYRITTYNAKAKAAKERGSNFTPKTVKSGGS
HS2 ----------MYG----TIFFMLFSNFWYHSYTKGKRLPRALQQNGAPGIAKVKAN
240 250 260 270
310
MAELO PKKPSKSKHI
X
HS2 280

FIG. 30B

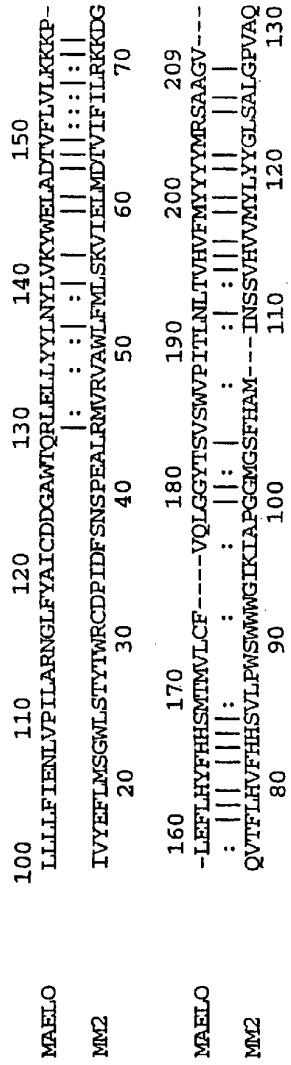
100 110 120 130 140 150
MAELO LLLLFTENLVFILARNGLFYAICDDGAWTQRLELLYYILNYLVKYWELADTVFLVLKKKP-
MM2 IVYEFLMSGWLSTYTWRCDPIDFSNSPEALRMVRVAWLFMLSKVIELMDTVIFILRKKDG
160 170 180 190 200 209
MAELO -LEFLHYFHHSMTMVLCF----VQLGGYTSVSWVPITLNLTVHVFMYYYYMRSAAGV---
MM2 QVTFLHVFHHSVLFWSWWWGIKIAPGGMGSFHAM---INSSVHVVMYLYYGLSALGPVAQ
210 220 230 240 250 260
MAELO -RIWWKQYLTTLQIVQFVL---DLGFIYF---CAYTYFAFTYFPWAPFNVGKCAGTEGAAL
MM2 PYLWWKKHMTAIQLIQFVLVSLHISQYYFMPSCNYQYPVIIHLLTW----------M
270 280 290 300 310
MAELO FGCGLLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVVKSGGSPKKPSKSKHI
MM2 YG----TIFFILFSNFWYHSYTKGKRLPRAVQQNGAPATTKVKAN
180 190 200 210

FIG.31

```
                30        40        50        60        70        80
MAELO           YELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFTLH
                                                  |||||:: |: | : ||:: |::|: :: |:
AI225632        NAFLDNMFGPRDSRVRGWFLLDSYLPTFILTTTYLLSIWLGNKYMKNRPALSLRGILTLY
                      20        30        40        50        60        70

                90       100       110       120       130       140
MAELO           NFLLTTASGSLLLLFIENLVPILARNGLFYAICDD----GAWTQRLELLYYLNYLVKYWE
                |: :|: |: :|: :|     : : :| :  |::     |    |: :    |: | |
AI225632        NLAITLLSAYMLVELI-----LSSWEGGYNLQCQNLDSAGEGDVRVAKVLVWYYFSKLVE
                      80        90       100       110       120

                150       160       170       180       190       200
MAELO           LADTVFLVLKKK--PLEFLHYFHHSMTMVLCFVQLGGYTSVSWVPTTLNLTVHVFMYYY
                : ||:|:||:||  : ||| :||:
AI225632        FLDTIFFVLRKKANQITFLHVYHHASMFNI
                  130       140       150
```

FIG.32

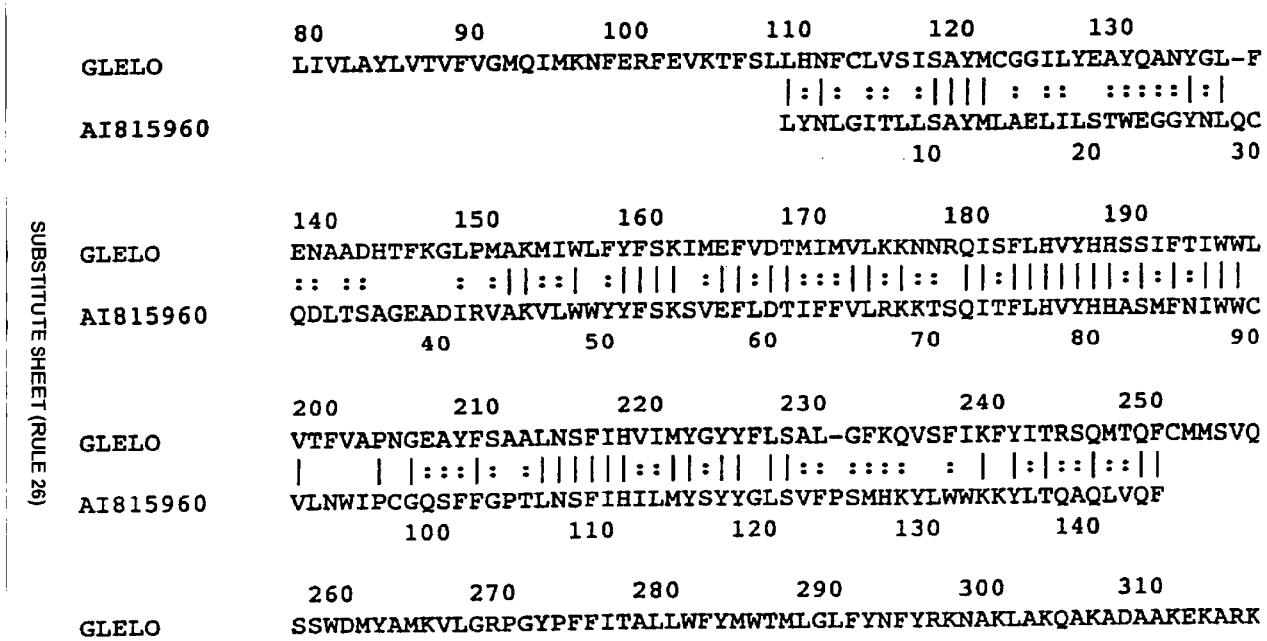

FIG.33

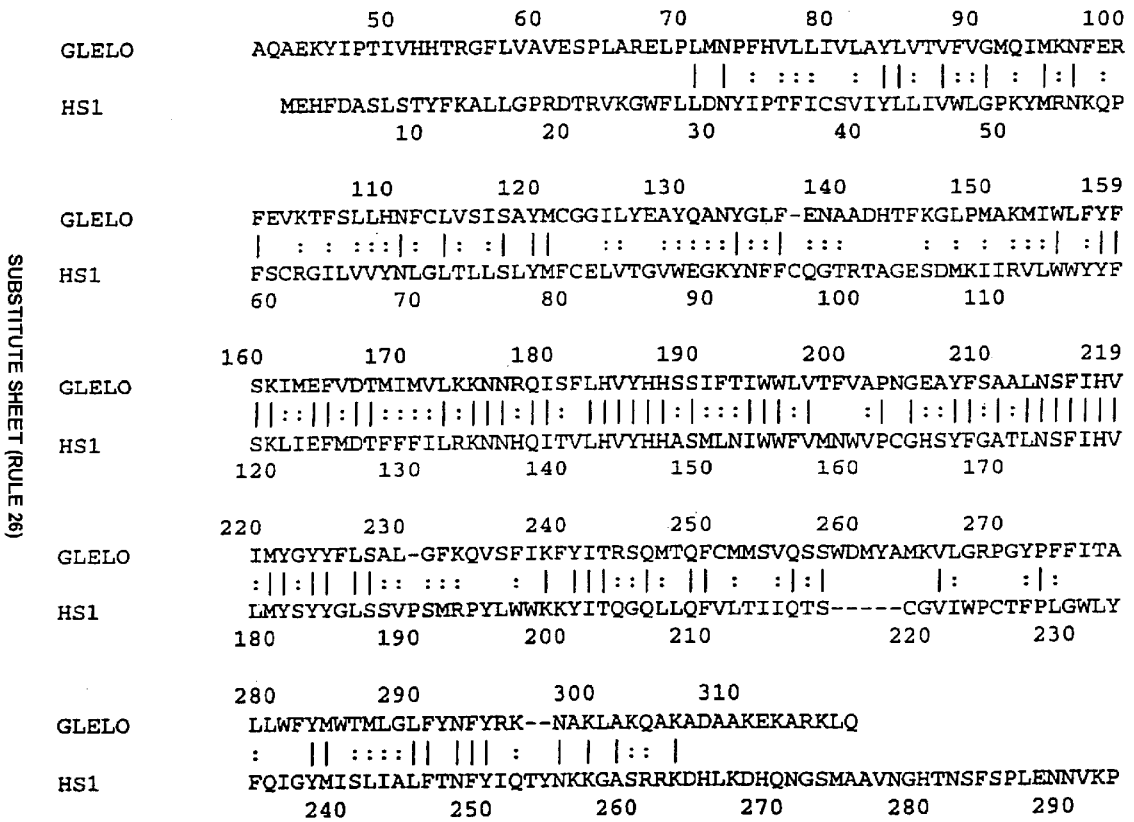

FIG.34

```
                140       150       160       170       180       190
GLELO           FENAADHTFKGLPMAKMIWLFYFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWW
                                                  ||:::::|:|  ::: ||| ||| ::: |
AC004050                                          DTIFIILRK--QKLIFLHWYHHITVLLYSW
                                                            10        20

                200       210       220       230       240       250
GLELO           LVTFVAPNGEAYFSAALNSFIHVIMYGYYFLSALGFKQVSFIKFYITRSQMTQFCMMSVQ
                      | ::|  ::|  :|::||:||  | | ||:    : ::|| ||:||: | |
AC004050        YSYKDMVAGGGWF-MTMNYGVHAVMYSYYALRAAGFRVSRKFAMFITLSQITQMLMGCVV
                30        40        50        60        70        80

                260       270       280       290       300       310
GLELO           SSWDMYAMKVLGRPGYPFFITALLW--FYMWTMLGLFYNFYRKN--AKLAKQAKADAAKE
                :  : |:   :  |   ::|  ::: ::| || :|: :  :|: | :||:
AC004050        NYLVFCWMQ--HDQCHSHF-QNIFWSSLMYLSYLVLFCHFFFEAYIGKMRKTTKAEX
                 90        100       110       120       130       140

GLELO           KARKLQ
```

FIG.35


80 90 100 110 120 130
GLELO LLIVLAYLVTVFVGMQIMKNFERFEVKTFSLLHNFCLVSISAYMCGGILYEAYQANYGLF
MM2 IVYNFSLVILSLYIVYEFLMSGWLSTYTWR
10 20 30
140 150 160 170 180 190
GLELO ENAAD--HTFKGLPMAKMIWLFYFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIW
MM2 CDPIDFSNSPEALRMVRVAWLFMLSKVIELMDTVIFILRKKDGQVTFLHVFHHSVLPWSW
40 50 60 70 80 90
200 210 220 230 240 250
GLELO WLVTFVAPNGEAYFSAALNSFIHVIMYGYYFLSALGFKQVSFI--KFYITRSQMTQFCMM
MM2 WWGIKIAPGGMGSFHAMINSSVHVVMYLYYGLSALGPVAQPYLWWKKHMTAIQLIQFVLV
100 110 120 130 140 150
260 270 280 290 300 309
GLELO SVQSSWDMYAMKVLGRPGYPFFITALLWFYMWTMLGLFYNF----YRKNAKLAKQAKADA
MM2 SLHIS-QYYFMPSCNYQ-YPVIIH-LIWMYGTIFFILFSNFWYHSYTKGKRLPRAVQQNG
160 170 180 190 200
310
GLELO AKEKARKLQ
MM2 APATTKVKAN
210

FIG.36

```
                50        60        70        80        90        100
GLELO           PTIVHHTRGFLVAVESPLARELPLMNPFHVLLIVLAYLVTVFVGMQIMKNFERFEVKTFS
                                                :::::::||:::::| : |||   : :: :
AI225632        NEVNAFLDNMFGPRDSRVRGWFLLDSYLPTFILTITYLLSIWLGNKYMKNRPALSLRGIL
                10        20        30        40        50        60

                110       120       130       140       150       160
GLELO           LLHNFCLVSISAYMCGGILYEAYQANYGLFENAADHTFKG-LPMAK-MIWLFYFSKIMEF
                 |:|: :: :|||||    ::  :::::|:|  :  | : :| : :|| ::| :||||:||
AI225632        TLYNLAITLLSAYMLVELILSSWEGGYNLQCQNLDSAGEGDVRVAKVLVW-YYFSKLVEF
                70        80        90        100       110       120

                  170       180       190       200       210       220
GLELO           VDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAALNSFIHVIMYGYY
                :|:::||:|:  ||:|||||||:|:|:|
AI225632        LDTIFFVLRKKANQITFLHVYHHASMFNI
                130       140       150
```

FIG.37

```
                20        30        40        50        60        70
GLELO   FMDLATAIGVRAAPYVDPLEAALVAQAEKYIPTIVHHTRGFLVAVESPLAREL-----PL
                                    : | :: :||: : : :|   |       |:
U97107                              MDTSMNFSRGLKMDLMQPYDFETFQDLRPF
                                   10        20        30

                    80        90        100       110       120       129
GLELO   MNPFHV--LLIVLAYLVTVFVGMQIMKNFERFEVKTFSLLHNFCLVSISAYMCGGILYEA
        :: : |  :|||::||: : ||:  |:: : |  ::   :| :| |: :|  : | : :
U97107  LEEYWVSSFLIVVVYLLLIVVGQTYMRTRKSFSLQRPLILWSFFLAIFS--ILGTLRMWK
                    40        50        60        70        80

                130       140       150       160       170       180
GLELO   YQAN----YGLFENAADHTFKGLPMAKMIW--LFYFSKIMEFVDTMIMVLKKNNRQISFL
        ::|:    ||  :::   :    ::::  |  ||  :||::|: ||  :::|:|  | : |:
U97107  FMATVMFTVGLKQTVCFAIYTDDAVVRF-WSFLFLLSKVVELGDTAFIILRK--RPLIFV
                90        100       110       120       130       140
```

FIG.38A

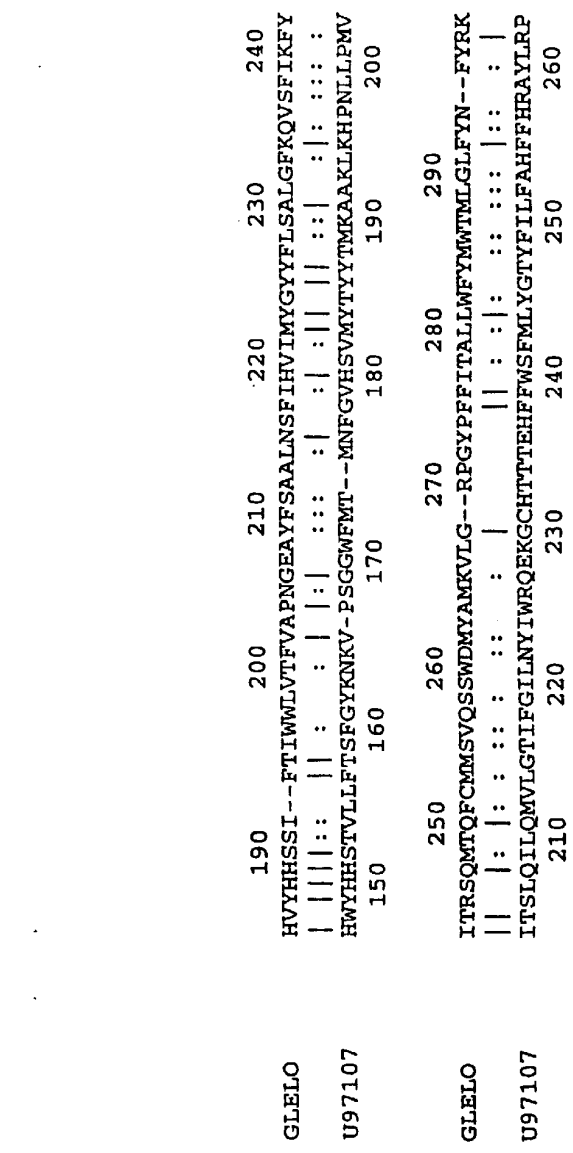
190 200 210 220 230 240
GLELO HVYHHSSI--FTIWWLVTFVAPNGEAYFSAALNSFIHVIMYGYYFLSALGFKQVSFIKFY
U97107 HWYHHSTVLLFTSFGYKNKV-PSGGWFMT--MNFGVHSVMYTYYTMKAAKLKHPNLLPMV
150 160 170 180 190 200
250 260 270 280 290
GLELO ITRSQMTQFCMMSVQSSWDMYAMKVLG--RPGYPFFITALLWFYMWTMLGLFYN--FYRK
U97107 ITSLQILQMVLGTIFGILNYIWRQEKGCHTTTEHFFWSFMLYGTYFILFAHFFHRAYLRP
210 220 230 240 250 260
300 310
GLELO NAKLAKQAKADAAKEKARKLQ
U97107 KGKVASKSQ
270

FIG.38B

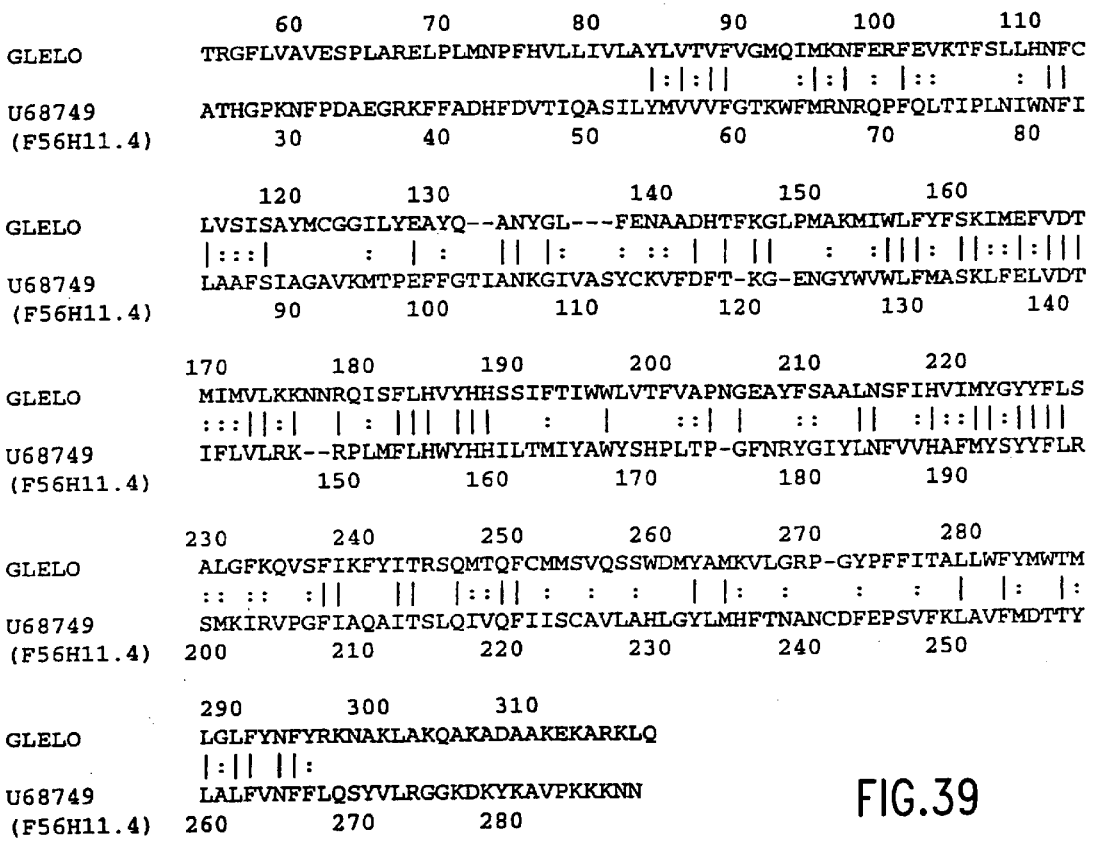

FIG.39

```
                10        20        30        40        50        59
MAELO           MAAAILDKVNFGIDQPFGIKLDTYFAQAYELVTGKSIDSFVFQEGVTPLSTQREVAMW-T
                           ::: : : :|:||  : :  :: ::  :|   ||   :: : :|:: :
U68749                  MAQHPLVQRLLDVKFDT---KRFVAIATHGPKNFPDAEGRKFFADHFDVTIQAS
(F56H11.4)                      10        20        30        40        50

                60        70        80        90        100       110
MAELO           ITYFVVIFGGRQIMKSQDAFKLK-PLFILHNFLLTIASGSLLLLFIENLVPILARNGLFY
                | |:|||:|| : :|:::: |:|  ||  |  ||:|: | : : : ::  :| :|:
U68749          ILYMVVVFGTKWFMRNRQPFQLTIPLNIW-NFILAAFSIAGAVKMTPEFFGTIANKGIVA
(F56H11.4)              60        70        80        90        100       110

                120       130       140       150       160       170
MAELO           AICDDGAWTQRLELLYYLNYLV-KYWELADTVFLVLKKKPLEFLHYFHHSMTMVLCFVQL
                : |   :|: :  :   ::: | :||:|||||:|:|| |||::||  :||: : :
U68749          SYCKVFDFTKGENGYWVWLFMASKLFELVDTIFLVLRKRPLMFLHWYHHILTMIYAWYSH
(F56H11.4)              120       130       140       150       160       170
```

FIG.40A

```
                180       190       200       210       220       230
MAELO           GGYTSVSWVPITLNLTVHVFMY-YYYMRSAAGVRI--WWKQYLTTLQIVQFVLDLGFIYF
                  : :   | ||:::|:|||  ||::||   :|:  :  | :|:|||||||::: : :
U68749          PLTPGFNRYGIYLNFVVHAFMYSYYFLRSMK-IRVPGFIAQAITSLQIVQFIISCAVLAH
(F56H11.4)          180       190       200       210       220

                    240       250       260       270       280
MAELO           CAYT-YFAFTYFPWAPNVGKCAGTEGAALFGCGLLSSYLLLFINFYRITY-----NAKAK
                  :|  :|: :   :  |:| | |        :|   :  ::|| ||:||:  :|     : | |
U68749          LGYLMHFTNANCDFEPSVFKLA------VF---MDTTYILALFVNFFLQSYVLRGGKDKYK
(F56H11.4)  230       240       250       260       270       280

                290       300       310
MAELO           AAKERGSNFTPKTVKSGGSPKKPSKSKHI
                |: :: :|
U68749          AVPKKKNN
(F56H11.4)
```

FIG.40B

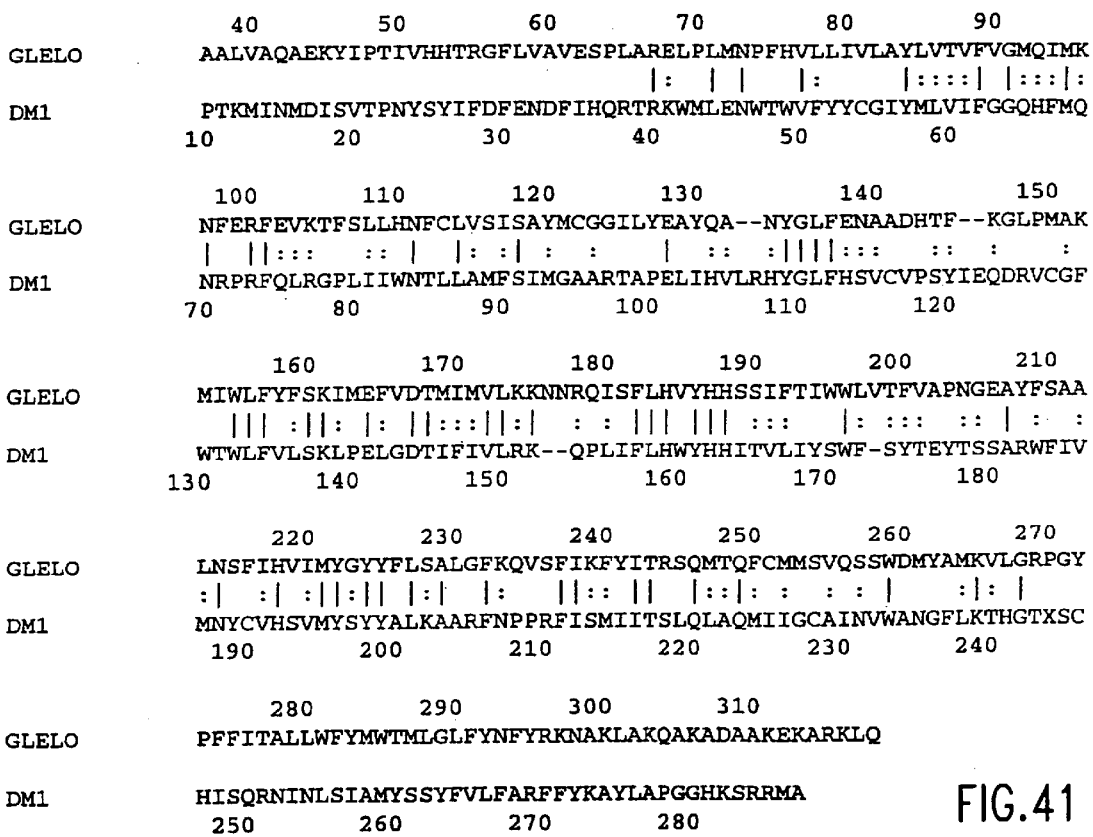

FIG.41

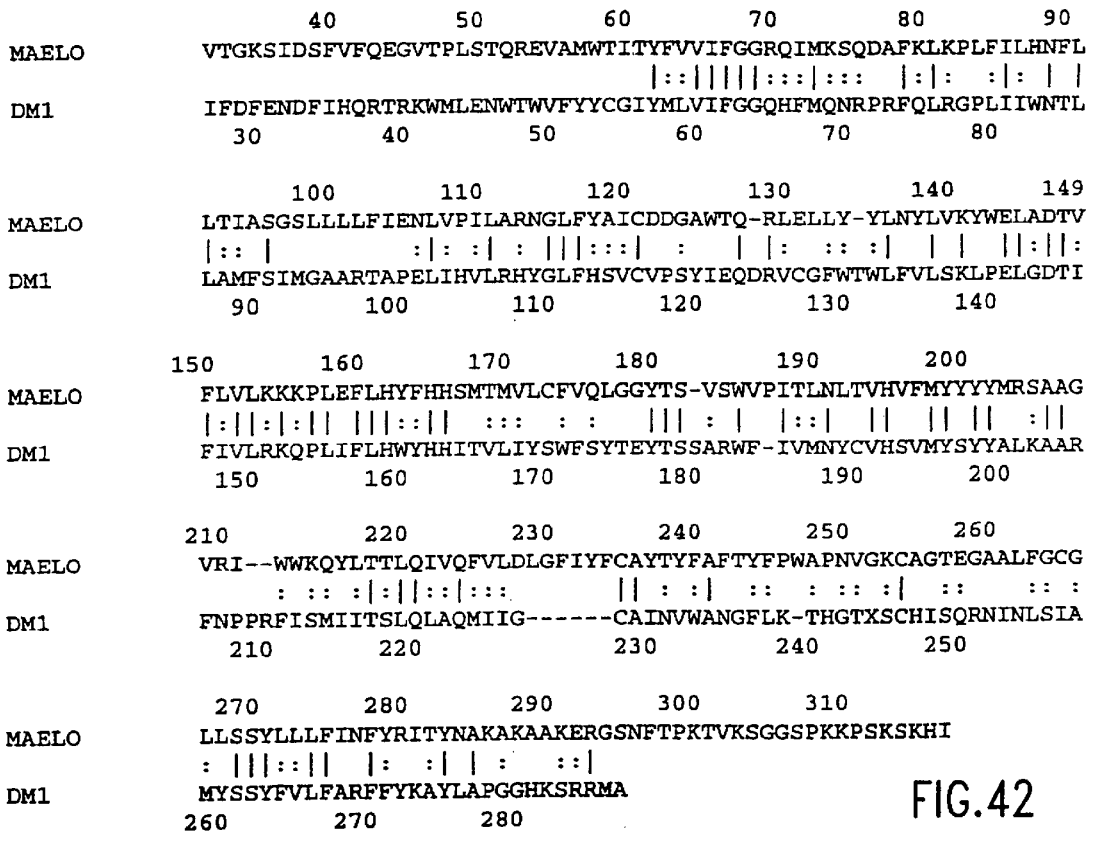

FIG.42

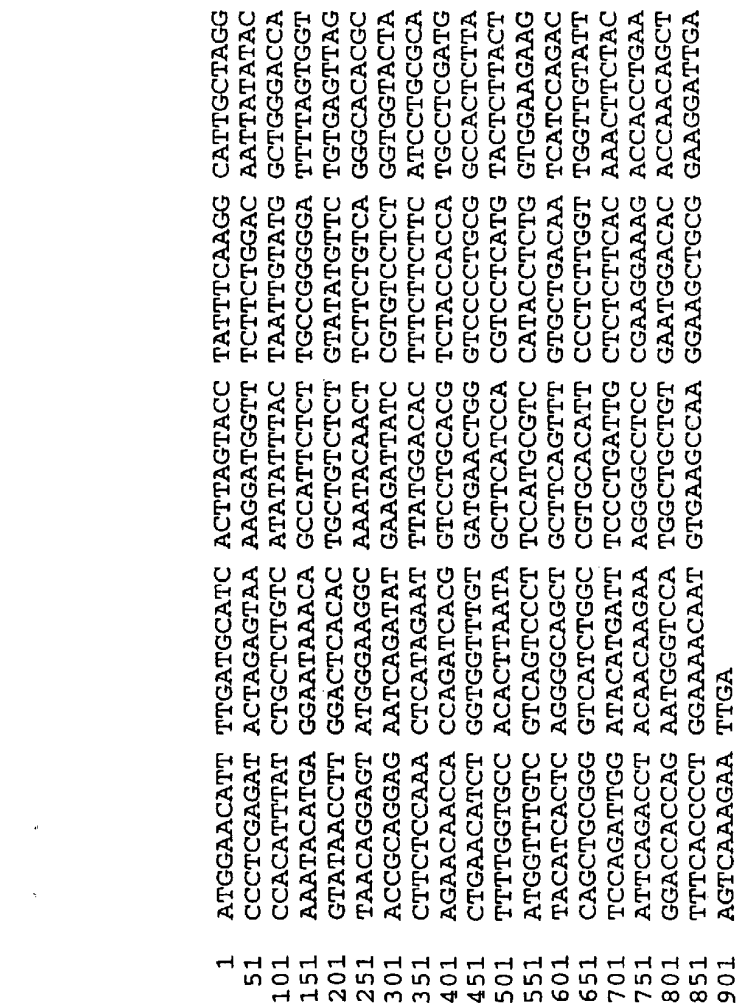
1 ATGGAACATT TTGATGCATC ACTTAGTACC TATTTCAAGG CATTGCTAGG
51 CCCTCGAGAT ACTAGAGTAA AAGGATGGTT TCTTCTGGAC AATTATATAC
101 CCACATTTAT CTGCTCTGTC ATATATTTAC TAATTGTATG GCTGGGACCA
151 AAATACATGA GGAATAAACA GCCATTCTCT TGCCGGGGGA TTTTAGTGGT
201 GTATAACCTT GGACTCACAC TGCTGTCTCT GTATATGTTC TGTGAGTTAG
251 TAACAGGAGT ATGGGAAGGC AAATACAACT TCTTCTGTCA GGGCACACGC
301 ACCGCAGGAG AATCAGATAT GAAGATTATC CGTGTCCTCT GGTGGTACTA
351 CTTCTCCAAA CTCATAGAAT TTATGGACAC TTTCTTCTTC ATCCTGCGCA
401 AGAACAACCA CCAGATCACG GTCCTGCACG TCTACCACCA TGCCTCGATG
451 CTGAACATCT GGTGGTTTGT GATGAACTGG GTCCCCCTGCG GCCACTCTTA
501 TTTTGGTGCC ACACTTAATA GCTTCATCCA CGTCCTCATG TACTCTTACT
551 ATGGTTTGTC GTCAGTCCCT TCCATGCGTC CATACCTCTG GTGGAAGAAG
601 TACATCACTC AGGGGCAGCT GCTTCAGTTT GTGCTGACAA TCATCCAGAC
651 CAGCTGCGGG GTCATCTGGC CGTGCACATT CCCTCTTGGT TGGTTGTATT
701 TCCAGATTGG ATACATGATT TCCCTGATTG CTCTCTTCAC AAACTTCTAC
751 ATTCAGACCT ACAACAAGAA AGGGGCCTCC CGAAGGAAAG ACCACCTGAA
801 GGACCACCAG AATGGGTCCA TGGCTGCTGT GAATGGACAC ACCAACAGCT
851 TTTCACCCCT GGAAAACAAT GTGAAGCCAA GGAAGCTGCG GAAGGATTGA
901 AGTCAAAGAA TTGA

FIG.43

```
  1 MEHFDASLST YFKALLGPRD TRVKGWFLLD NYIPTFICSV IYLLIVWLGP
 51 KYMRNKQPFS CRGILVVYNL GLTLLSLYMF CELVTGVWEG KYNFFCQGTR
101 TAGESDMKII RVLWWTYFSK LIEFMDTFFF ILRKNNHQIT VLHVYHHASM
151 LNIWWFVMNW VPCGHSYFGA TLNSFIHVLM YSYYGLSSVP SMRPYLWWKK
201 YITQGQLLQF VLTIIQTSCG VIWPCTFPLG WLYFQIGYMI SLIALFTNFY
251 IQTYNKKGAS RRKDHLKDHQ NGSMAAVNGH TNSFSPLENN VKPRKLRKD*
```

FIG.44

Host (plasmid)	334(pYX242)	334(pRAE-58-A1)	334(pYX242)	334(pRAE-58-A1)
Added substrate	25 µM GLA	25 µM GLA	25 µM AA	25 µM AA
Fatty acid	%total fatty acid	%total fatty acid	%total fatty acid	%total fatty acid
C18:3n-6	4.40	2.71	0.03	0.04
C20:3n-6	0.09	**(50.34%)* 2.75**	0.02	0.02
C20:4n-6			7.84	3.97
C224n-6			ND	**(23.37%)* 1.21**
C16:1n-7	41.11	34.72	41.49	35.07
C18:1n-7	1.85	**11.33**	2.01	**11.57**
C20:1n-7	0.04	**1.48**	0.04	**1.62**
C18:1n-9	15.60	15.66	15.16	14.57
C20:1n-9	0.06	**0.22**	0.06	**0.23**
C18:1n-5	0.11	**0.62**	0.12	**0.58**
Total Lipid	370	969	359	514
*% conversion=product/(substrate+product)				

FIG.45

```
   1 ATGGCTCAGC ATCCGCTCGT TCAACGGCTT CTCGATGTCA AATTCGACAC
  51 GAAACGATTT GTGGCTATTG CTACTCATGG GCCAAAGAAT TTCCCTGACG
 101 CAGAAGGTCG CAAGTTCTTT GCTGATCACT TTGATGTTAC TATTCAGGCT
 151 TCAATCCTGT ACATGGTCGT TGTGTTCGGA ACAAAATGGT TCATGCGTAA
 201 TCGTCAACCA TTCCAATTGA CTATTCCACT CAACATCTGG AATTTCATCC
 251 TCGCCGCATT TTCCATCGCA GGAGCTGTCA AAATGACCCC AGAGTTCTTT
 301 GGAACCATTG CCAACAAAGG AATTGTCGCA TCCTACTGCA AAGTGTTTGA
 351 TTTCACGAAA GGAGAGAATG GATACTGGGT GTGGCTCTTC ATGGCTTCCA
 401 AACTTTTCGA ACTTGTTGAC ACCATCTTCT TGGTTCTCCG TAAACGTCCA
 451 CTCATGTTCC TTCACTGGTA TCACCATATT CTCACCATGA TCTACGCCTG
 501 GTACTCTCAT CCATTGACCC CAGGATTCAA CAGATACGGA ATTTATCTTA
 551 ACTTTGTCGT CCACGCCTTC ATGTACTCTT ACTACTTCCT TCGCTCGATG
 601 AAGATTCGCG TGCCAGGATT CATCGCCCAA GCTATCACAT CTCTTCAAAT
 651 CGTTCAATTC ATCATCTCTT GCGCCGTTCT TGCTCATCTT GGTTATCTCA
 701 TGCACTTCAC CAATGCCAAC TGTGATTTCG AGCCATCAGT ATTCAAGCTC
 751 GCAGTTTTCA TGGACACAAC ATACTTGGCT CTTTTCGTCA ACTTCTTCCT
 801 CCAATCATAT GTTCTCCGCG GAGGAAAAGA CAAGTACAAG GCAGTGCCAA
 851 AGAAGAAGAA CAACTAA
```

FIG.46

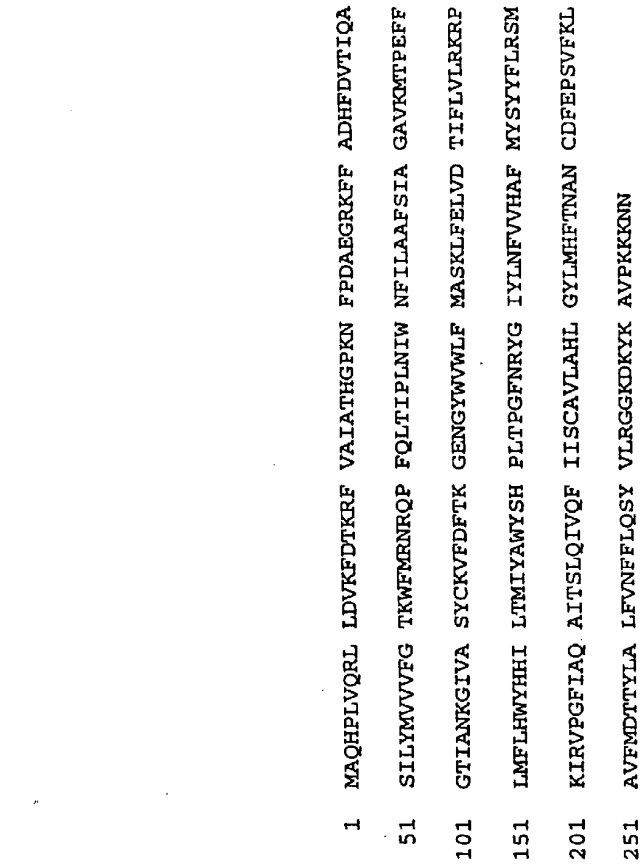
1 MAQHPLVQRL LDVKFDTKRF VAIATHGPKN FPDAEGRKFF ADHFDVTIQA
51 SILYMVVVFG TKWFMRNRQP FQLTIPLNIW NFILAAFSIA GAVKMTPEFF
101 GTIANKGIVA SYCKVFDFTK GENGYWVWLF MASKLFELVD TIFLVLRKRP
151 LMFLHWYHHI LTMIYAWYSH PLTPGFNRYG IYLNFVVHAF MYSYYFLRSM
201 KIRVPGFIAQ AITSLQIVQF IISCAVLAHL GYLMHFTNAN CDFEPSVFKL
251 AVFMDTTYLA LFVNFFLQSY VLRGGKDKYK AVPKKKNN

FIG.47

Host (plasmid)	334(pYX242)	334(pRET-21)	334(pRET-22)
Added Substrates	50 µM GLA +	50 µM GLA +	50 µM GLA +
	50 µM AA	50 µM AA	50 µM AA
Fatty Acid	%total fatty acid	%total fatty acid	%total fatty acid
C16:0	9.22	12.46	9.9
C16:1	0.09	0.18	0.13
C18:0	1.46	2.41	1.49
C18:1n-9	4.03	4.92	3.91
C18:3n-6	10.02	11.89	8.69
C20:3n-6	**(1.28%)* 0.13**	**(11.1%)* 1.48**	**(19.4%)* 2.09**
C20:4n-6	46.98	28.87	35.25
C22:4n-6	0	0	0
Total lipid (mg)	212	174	187
*% conversion=product/(substrate+product)			

FIG.48

```
1   ATGAACATGT CAGTGTTGAC TTTACAAGAA TATGAATTCG AAAAGCAGTT
51  CAACGAGAAT GAAGCCATCC AATGGATGCA GGAAAACTGG AAGAAATCTT
101 TCCTGTTTTC TGCTCTGTAT GCTGCCTTTA TATTCGGTGG TCGGCACCTA
151 ATGAATAAAC GAGCAAAGTT TGAACTGAGG AAGCCATTAG TGCTCTGGTC
201 TCTGACCCTT GCAGTCTTCA GTATATTCGG TGCTCTTCGA ACTGGTGCTT
251 ATATGGTGTA CATTTTGATG ACCAAAGGCC TGAAGCAGTC AGTTTGTGAC
301 CAGGGTTTTT ACAATGGACC TGTCAGCAAA TTCTGGGCTT ATGCATTTGT
351 GCTAAGCAAA GCACCCGAAC TAGGAGATAC AATATTCATT ATTCTGAGGA
401 AGCAGAAGCT GATCTTCCTG CACTGGTATC ACCACATCAC TGTGCTCCTG
451 TACTCTTGGT ACTCCTACAA AGACATGGTT GCCGGGGGAG GTTGGTTCAT
501 GACTATGAAC TATGGCGTGC ACGCCGTGAT GTACTCTTAC TATGCCTTGC
551 GGGCGGCAGG TTTCCGAGTC TCCCGGAAGT TTGCCATGTT CATCACCTTG
601 TCCCAGATCA CTCAGATGCT GATGGGCTGT GTGGTTAACT ACCTGGTCTT
651 CTGCTGGATG CAGCATGACC AGTGTCACTC TCACTTTCAG AACATCTTCT
701 GGTCCTCACT CATGTACCTC AGCTACCTTG TGCTCTTCTG CCATTTCTTC
751 TTTGAGGCCT ACATCGGCAA AATGAGGAAA ACAACGAAAG CTGAATAG
```

FIG.49

```
  1  MNMSVLTLQE YEFEKQFNEN EAIQWMQENW KKSFLFSALY AAFIFGGRHL

 51  MNKRAKFELR KPLVLWSLTL AVFSIFGALR TGAYMVYILM TKGLKQSVCD

101  QGFYNGPVSK FWAYAFVLSK APELGDTIFI ILRKQKLIFL HWYHHITVLL

151  YSWYSYKDMV AGGGWFMTMN YGVHAVMYSY YALRAAGFRV SRKFAMFITL

201  SQITQMLMGC VVNYLVFCWM QHDQCHSHFQ NIFWSSLMYL SYLVLFCHFF

251  FEAYIGKMRK TTKAE*
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FIG.50