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Molecular cloning and sequencing of DNA complementary to chicken liver fatty acid synthase mRNA

(multienzyme complex/cDNA clones/protein sequence)

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ABSTRACT **The cDNA corresponding to 4.18 kilobases (kb) of the mRNA of chicken liver fatty acid synthase has been cloned and sequenced. The cDNA corresponds to the 3′ end of the mRNA and consists of a 1.87-kb noncoding tail and a 2.31-kb region encoding 769 amino acids of the C terminus of the enzyme. The thioesterase at the C terminus, preceded by the acyl carrier protein, can be identified from known amino acid sequences. However, the identity of the enzymes N terminal to the acyl carrier protein could not be ascertained. The partial amino acid sequence of the chicken liver fatty acid synthase shows >70% similarity with the rat mammary gland enzyme.**

Animal fatty acid synthases are multienzyme complexes that catalyze the synthesis of palmitic acid from acetyl CoA and malonyl CoA with NADPH as a reducing agent:

$$\text{acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 14\ \text{H}^+ \rightarrow$$
$$\text{palmitic acid} + 8\ \text{CoA} + 14\ \text{NADP}^+ + 6\ \text{H}_2\text{O} + 7\ \text{CO}_2$$

The kinetics and stereochemistry of many of the elementary steps in the reaction mechanism have been studied (cf. ref. 1). An acetyl group is first transferred to the enzyme from acetyl CoA; a malonyl group is then condensed with the acetyl to form a β-ketoacyl intermediate, which is reduced by NADPH to a β-hydroxy intermediate. This β-hydroxy intermediate is dehydrated to form a carbon-carbon double bond that is reduced by a second NADPH to give a saturated enzyme-bound fatty acid. The sequence of reactions initiated by the addition of malonyl to the fatty acid is repeated six more times until enzyme-bound palmitate is formed, and palmitate is released from the enzyme by a thioesterase.

The structure of the fatty acid synthase has been extensively studied. The enzyme is composed of two identical polypeptides of M_r 250,000 (cf. ref. 2). A functional map of the enzyme has been proposed on the basis of limited proteolysis experiments (2–4). Three primary functional domains exist: domain I (M_r, 127,000) contains acetyl and malonyl transacylases and β-ketoacyl synthase; domain II (M_r, 107,000) contains dehydratase, β-ketoacyl reductase, and enoyl reductase; and domain III (M_r, 33,000) is the thioesterase. The thioesterase is located at the C terminus of the polypeptide and has considerable segmental flexibility (5). Amino acid sequences in active site regions of fatty acid synthase from chicken liver have been obtained (6).

Attempts have been made to clone the gene for animal fatty acid synthase (7–10). A partial genomic cloning of the gene for the chicken liver enzyme (9), which covers ≈150 amino acids near the C terminus, reveals that many introns exist between exons. In this study, cDNA clones have been isolated and sequenced that encode more than one-third of the chicken liver fatty acid synthase polypeptide. The complete 3′ noncoding region of the enzyme mRNA also has been cloned and sequenced.*

MATERIALS AND METHODS

Chemicals. RNase A, DNase I, T4 DNA ligase, T4 DNA kinase, and restriction enzymes were obtained from Bethesda Research Laboratories. [α-^{32}P]dATP and [γ-^{32}P]ATP were from ICN. All other chemicals were high purity commercial grades. Oligonucleotides were synthesized with a 380B DNA synthesizer from Applied Biosystems (Foster City, CA) in the Cornell University Biotechnology Program facility. When necessary, the synthesized oligonucleotide was separated from impurities by passage through an anion-exchange column. The DNA sequencing kit (Sequenase) was from United States Biochemical (Cleveland).

cDNA Library Screening. An adult leghorn rooster liver cDNA library was purchased from Clontech Laboratories (Palo Alto, CA). The cDNA is in bacteriophage λgt11, and the cloning site is *Eco*RI. About 66% of the library phage has inserts with an average size of 0.9 kilobase (kb).

The chicken liver cDNA library was screened with synthesized oligonucleotides. Approximately 5×10^5 phage were plated out per 150 mm NZ agar plate for the first screening. The transfer of the phage DNA to nitrocellulose filters was done according to Maniatis *et al.* (11). Duplicate filters were made for each plate. The oligonucleotides were designed according to protein sequences reported by Ranganna *et al.* (9). Two 20-mers were made and purified. One of the oligonucleotides (END) corresponds to the C terminus of the thioesterase domain, and the other (MID) corresponds to the middle of the thioesterase. End-labeling of the oligonucleotides and hybridization of the nitrocellulose filters were performed according to Woods (12). The filters were washed in 0.9 M NaCl/90 mM sodium citrate solution at 64°C for 2 min before autoradiography. The pseudopositive signals were picked up and subjected to further screening.

Subcloning of cDNA Fragments. cDNA fragments were generated by *Eco*RI digestion of cDNA containing λgt11 phage DNA. The cDNA fragments were separated from the digestion mixture by gel electrophoresis in low melting point agarose. The correct DNA band was sliced out, melted at 65°C, and extracted with phenol. The purified cDNA fragments were cloned into the PUC19 plasmid for generating large quantities of DNA or into bacteriophage M13mp11 for the preparation of single-stranded DNA. PUC19 or M13mp11RF was digested with *Eco*RI and then ligated with the cDNA fragments using T4 DNA ligase at 16°C.

Sequencing of cDNA. The cDNA was enzymatically sequenced either in single-stranded M13mp11 or in PUC19 with the Sequenase kit. Template DNA was prepared as suggested

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*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03860).

by the sequencing kit manufacturer. Both strands of cDNA fragments were sequenced with the universal primer. Oligonucleotides (16-mers) were then synthesized according to sequence information obtained and used directly as primers for further sequencing without purification on an anion-exchange column.

RESULTS AND DISCUSSION

Approximately one million independent clones from the chicken liver cDNA library were screened with synthesized oligonucleotides. Thirteen pseudopositive signals were obtained. Further screening and analysis showed that three λgt11 clones (numbers 3, 10, and 13) could hybridize with both MID and END oligonucleotides (which correspond to the middle and C terminus regions of the thioesterase). *Eco*RI digestion and subsequent gel electrophoresis showed that the insert size for the clones was 4200, 2800, and 1400 base pairs (bp), respectively. Numbers 3 and 13 have one internal *Eco*RI restriction site. Clone 10 does not have this site. *Eco*RI restriction digestion generated 2800- and 1400-bp fragments from clone 3, and 950- and 450-bp fragments from clone 13.

Southern analysis (13) of the *Eco*RI-digested products showed that only clone 10 (2800 bp), the 2800-bp fragment of clone 3, and the 950-bp fragment of clone 13 could hybridize with both END and MID oligonucleotides, which suggests that the 1400-bp fragment of clone 3 and the 450-bp fragment of clone 13 are in the 3′ noncoding region of the fatty acid synthase mRNA. Partial DNA sequencing analysis showed that the 2800-bp fragment of clone 3 is similar to clone 10. The 1400-bp fragment of clone 3 shows a poly(A) tail at one end, which suggests that this clone contains the end of the 3′ noncoding region for the fatty acid synthase mRNA.

Both strands of clones 3 and 10 were sequenced with the sequencing strategy shown in Fig. 1. The complete DNA sequence and deduced amino acid sequence are summarized in Fig. 2. The sequence results indicate that the 2800-bp fragment of clone 3 is almost identical to clone 10, except that clone 3 has an extra 24 nucleotides (positions 1798–1821), as indicated by the box in Fig. 2. The amino acid sequence coded by nucleotides 1168–1242 matches exactly the sequence obtained for the region of the protein containing 4′-phosphopantetheine (6). The region in parentheses encoded by nucleotides 1108–1371 is the acyl carrier protein region and is identical to the amino acid sequence obtained through sequencing of the protein (14). The region in parentheses encoded by nucleotides 1372–2307 is believed to be the thioesterase domain and matches most of the amino acid sequence reported by Ranganna *et al.* (9). Thus, the cDNA clones isolated correspond to the 3′ end of the fatty acid synthase mRNA.

Of the 4180 nucleotides sequenced, 2307 nucleotides are in the coding region of the mRNA. This encodes 769 amino acids, which corresponds to a M_r of 84,605, or more than one-third of the total enzyme polypeptide. The size of the 3′ noncoding region (1800 bp) is slightly longer than the previous estimate of 1700 bp obtained by S1 nuclease digestion (9). The less commonly encountered polyadenylylation signal ATTAAA (15) is located 21 nucleotides upstream from the poly(A) tail.

The 369 amino acids encoded by nucleotides 1–1107 cannot be assigned to specific enzymes in chicken liver fatty acid synthase. Based on the functional map proposed by Wakil *et al.* (2), the functional domain next to the acyl carrier protein should be the ketoacyl reductase. However, the lack of amino acid sequence information for the ketoacyl reductase domain prevents a definitive interpretation.

The sequence of 593 amino acids at the C terminus of the rat mammary gland fatty acid synthase has been deduced from cDNA cloning (10, 16). Comparison between the corresponding sequences of rat and chicken fatty acid synthase indicates a very great similarity in both amino acid and DNA sequences. More than 44% of the nucleotides between 534 and 2309 have sequences 3 nucleotides or longer matching those of the rat enzyme. Of the 593 amino acids compared, 408 amino acids match exactly, as indicated in Fig. 2 by underlines in the amino acid sequence. The longest conserved sequence is 28 amino acids long (encoded by nucleotides 2173–2256). Mostly conservative substitutions are found at the mismatched positions. However, the rat fatty acid synthase has two extra amino acids after nucleotide positions 960 and 1122. Comparison of the 3′ noncoding region for the fatty acid synthase mRNA from rat and chicken shows no obvious homology.

Despite the fact that chicken and rat are evolutionarily distant organisms, a high degree of homology (>70% for amino acids and >44% for DNA) exists between the rat and chicken protein and DNA sequences of the enzyme. This suggests that fatty acid synthase is a very conserved enzyme. A long string of amino acid mismatches (encoded by nucleotides 1360–1398) exists in the region where functional domains of thioesterase and acyl carrier protein are connected to each other. Another long string of amino acid mismatches (encoded by nucleotides 1105–1122) connects the acyl carrier protein to the next functional domain. The positions of these strings of mismatches suggest that the genes for the functional domains of the enzyme were originally separated [as in the bacterial fatty acid synthase system, which contains seven enzymes that are not covalently linked (2)], and these genes were connected to each other during evolution to improve the efficiency of fatty acid synthesis. During the process of connecting enzymes to each

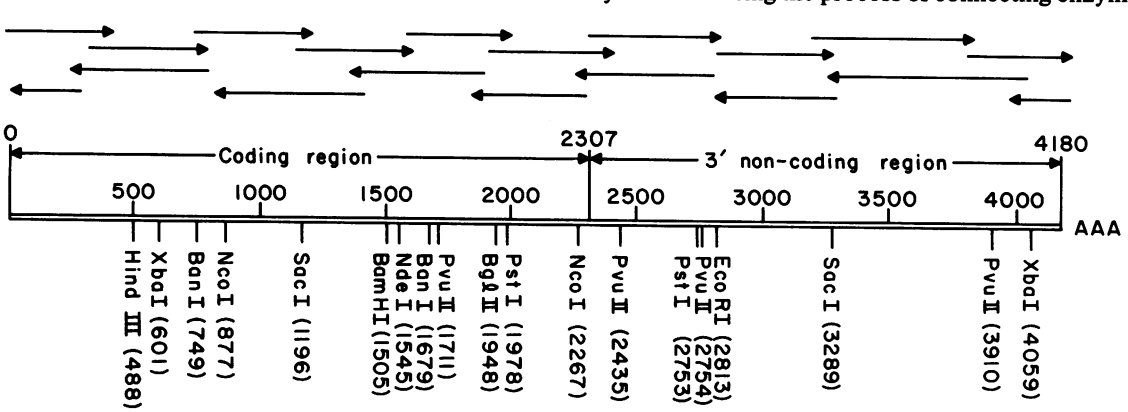

FIG. 1. Restriction map and sequencing strategy for cDNA clones 3 and 10. Arrows indicate the directions and positions of synthetic oligonucleotides used in priming. The coding region has been sequenced for both clones 3 and 10.

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                    18                  36                  54                  72                  90
TTG GCA GAA GAG AAG CTC CAA GCC AGT TTG CGT TGT CTT GCT CAA CAT GGG CGC TTC TTG GAA ATA GGC AAA TTT GAT CTA TCA AAC AAC
Leu Ala Glu Glu Lys Leu Gln Ala Ser Leu Arg Cys Leu Ala Gln His Gly Arg Phe Leu Glu Ile Gly Lys Phe Asp Leu Ser Asn Asn
                   108                 126                 144                 162                 180
AGC CAG CTT GGA ATG GCT CTT TTC CTC AAG AAT GTG GCG TTT CAT GGA ATC CTG CTG GAT TCA ATC TTT GAG GAA GGA AAC CAA GAG TGG
Ser Gln Leu Gly MET Ala Leu Phe Leu Lys Asn Val Ala Phe His Gly Ile Leu Leu Asp Ser Ile Phe Glu Glu Gly Asn Gln Glu Trp
                   198                 216                 234                 252                 270
GAG GTG GTA TCA GAG TTG TTG ACA AAA GGC ATA AAA GAT GGT GTG GTA AAG CCC CTG AGA ACC ACA GTC TTC GGT AAA GAA GAG GTA GAA
Glu Val Val Ser Glu Leu Leu Thr Lys Gly Ile Lys Asp Gly Val Val Lys Pro Leu Arg Thr Thr Val Phe Gly Lys Glu Glu Val Glu
                   288                 306                 324                 342                 360
GCT GCC TTC AGG TTC ATG GCG CAA GGA AAA CAT ATT GGC AAA GTT ATG ATC AAG ATC CAA GAA GAG GAG AAG CAA TAT CCT TTA AGG TCT
Ala Ala Phe Arg Phe MET Ala Gln Gly Lys His Ile Gly Lys Val MET Ile Lys Ile Gln Glu Glu Glu Lys Gln Tyr Pro Leu Arg Ser
                   378                 396                 414                 432                 450
GAA CCA GTA AAA CTC TCT GCC ATC TCC CGA ACT TCC TGC CCA CCT ACC AAG TCT TAC ATC ATC ACA GGG GGC CTA GGA GGA TTT GGG CTT
Glu Pro Val Lys Leu Ser Ala Ile Ser Arg Thr Ser Cys Pro Pro Thr Lys Ser Tyr Ile Ile Thr Gly Gly Leu Gly Gly Phe Gly Leu
                   468                 486                 504                 522                 540
GAG TTG GCA CAG TGG CTA ATT GAG AGA GGA GCA CAG AAG CTT GTA CTG ACA TCT CGA TCT GGC ATA CGA ACT GGC TAC CAG GCT AAA TGT
Glu Leu Ala Gln Trp Leu Ile Glu Arg Gly Ala Gln Lys Leu Val Leu Thr Ser Arg Ser Gly Ile Arg Thr Gly Tyr Gln Ala Lys Cys
                   558                 576                 594                 612                 630
GTT AGA GAA TGG AAG GCG CTG GGA ATC CAA GTG TTG GTC TCT ACC AGT GAT GTT GGA ACT CTA GAA GGA ACG CAG CTT TTG ATA GAA GAG
Val Arg Glu Trp Lys Ala Leu Gly Ile Gln Val Leu Val Ser Thr Ser Asp Val Gly Thr Leu Glu Gly Thr Gln Leu Leu Ile Glu Glu
                   648                 666                 684                 702                 720
GCT TTG AAG CTC GGA CCA GTT GGG GGC ATC TTT AAT TTG GCT GTG GTC CTT AAA GAT GCC ATG ATT GAA AAT CAG ACC CCG GAA TTA TTC
Ala Leu Lys Leu Gly Pro Val Gly Gly Ile Phe Asn Leu Ala Val Val Leu Lys Asp Ala MET Ile Glu Asn Gln Thr Pro Glu Leu Phe
                   738                 756                 774                 792                 810
TGG GAG GTC AAC AAG CCC AAG TAT TCA GGC ACC CTT CAT TTG GAC TGG GTG ACT CGT AAG AAG TGC CCA GAC CTG GAC TAT TTT GTT GTA
Trp Glu Val Asn Lys Pro Lys Tyr Ser Gly Thr Leu His Leu Asp Trp Val Thr Arg Lys Lys Cys Pro Asp Leu Asp Tyr Phe Val Val
                   828                 846                 864                 882                 900
TTC TCC TCT GTA AGC TGT GGA AGA GGA AAT GCT GGG CAA AGT AAT TAT GGC TTT GCT AAT TCT GCC ATG GAG CGT ATC TGT GAG CAG CGG
Phe Ser Ser Val Ser Cys Gly Arg Gly Asn Ala Gly Gln Ser Asn Tyr Gly Phe Ala Asn Ser Ala MET Glu Arg Ile Cys Glu Gln Arg
                   918                 936                 954                 972                 990
CAT CAC GAT GGG CTC CCA GGC CTG GCA GTC CAG TGG GGA GCC ATT GGT GAT GTG GGC ATC CTG AAG GCA ATG GGA AAC AGG GAG GTT GTG
His His Asp Gly Leu Pro Gly Leu Ala Val Gln Trp Gly Ala Ile Gly Asp Val Gly Ile Leu Lys Ala MET Gly Asn Arg Glu Val Val
                  1008                1026                1044                1062                1080
ATT GGG GGA ACC GTT CTC CAG CAA ATC AGC TCC TGC CTG GAG GTG CTC GAT ATG TTC CTG AAT CAA CCT CAT CCT GTT ATG TCC AGT TTT
Ile Gly Gly Thr Val Leu Gln Gln Ile Ser Ser Cys Leu Glu Val Leu Asp MET Phe Leu Asn Gln Pro His Pro Val MET Ser Ser Phe
                  1098                1116                1134                1152                1170
GTC CTA GCA GAG AAG GTC TCT GTG AAA AGT GAA GGA GGA AGT CAA CGG GAT CTT GTA GAA GCT GTT GCT CAT ATC CTT GGT GTT CGT GAC
Val Leu Ala Glu Lys Val Ser Val Lys Ser Glu Gly Gly Ser Gln Arg Asp Leu Val Glu Ala Val Ala His Ile Leu Gly Val Arg Asp
                  1188                1206                1224                1242                1260
GTG AGC AGT CTG AAT GCT GAG AGC TCC CTA GCA GAC TTG GGC CTG GAT TCC TTG ATG GGT GTG GAG GTG CGC CAG ACG CTG GAG AGA GAC
Val Ser Ser Leu Asn Ala Glu Ser Ser Leu Ala Asp Leu Gly Leu Asp Ser Leu MET Gly Val Glu Val Arg Gln Thr Leu Glu Arg Asp
                  1278                1296                1314                1332                1350
TAC GAC ATC GTA ATG ACC ATG AGG GAG ATC CGA CTC CTC ACC ATC AAC AAA CTG CGT GAA CTG TCC TCC AAG ACT GGG ACA GCA GAG GAG
Tyr Asp Ile Val MET Thr MET Arg Glu Ile Arg Leu Leu Thr Ile Asn Lys Leu Arg Glu Leu Ser Ser Lys Thr Gly Thr Ala Glu Glu
                  1368                1386                1404                1422                1440
CTG AAG CCA TCA CAA GTG TTG AAG ACA GGC CCA GGT GAG CCT CCA AAA CTG GAT TTG AAC AAC TTG CTG GTG AAT CCA GAA GGG CCA ACG
Leu Lys Pro Ser Gln Val Leu Lys Thr Gly Pro Gly Glu Pro Pro Lys Leu Asp Leu Asn Asn Leu Leu Val Asn Pro Glu Gly Pro Thr
                  1458                1476                1494                1512                1530
ATT ACC CGT CTC AAT GAA GTT CAG AGC ACA GAA CGC CCT CTT TTC CTT GTT CAC CCC ATT GAG GGA TCC ATT GCA GTC TTC TAT ACT CTT
Ile Thr Arg Leu Asn Glu Val Gln Ser Thr Glu Arg Pro Leu Phe Leu Val His Pro Ile Glu Gly Ser Ile Ala Val Phe Tyr Thr Leu
                  1548                1566                1584                1602                1620
GCC TCC AAA CTT CAT ATG CCC TGC TAT GGA CTC CAG TGC ACA AAA GCT GCT CCC TTG GAC AGC ATA CAG AGC CTG GCA TCC TAT TAT ATT
Ala Ser Lys Leu His MET Pro Cys Tyr Gly Leu Gln Cys Thr Lys Ala Ala Pro Leu Asp Ser Ile Gln Ser Leu Ala Ser Tyr Tyr Ile
                  1638                1656                1674                1692                1710
GAC TGT ATG AAG CAG ATA CAG CCT GAA GGA CCT TAT CGC ATT GCT GGA TAC TCT TTT GGT GCC TGC GTA GCC TTT GAA ATG TGC TCC CAG
Asp Cys MET Lys Gln Ile Gln Pro Glu Gly Pro Tyr Arg Ile Ala Gly Tyr Ser Phe Gly Ala Cys Val Ala Phe Glu MET Cys Ser Gln
                  1728                1746                1764                1782                1800
CTG CAA GCA CAA CAA AAT GCT TCC CAT GCA CTC AAC AGT TTA TTC CTC TTT GAT GGG TCT CAT TCC TTT GTG GCA GCA TAC ACT CAG TGT
Leu Gln Ala Gln Gln Asn Ala Ser His Ala Leu Asn Ser Leu Phe Leu Phe Asp Gly Ser His Ser Phe Val Ala Ala Tyr Thr Gln Cys
                  1818                1836                1854                1872                1890
TTT TCC TTT TCT CTT TTT CAG AGC TAC AGA GCA AAG CTG ACC CAA GGA AAT GAG GCT GCG TTG GAG ACA GAA GCA CTG TGT GCC TTT GTT
Phe Ser Phe Ser Leu Phe Gln Ser Tyr Arg Ala Lys Leu Thr Gln Gly Asn Glu Ala Ala Leu Glu Thr Glu Ala Leu Cys Ala Phe Val
                  1908                1926                1944                1962                1980
CAG CAG TTT ACA GGC ATT GAA TAC AAT AAG TTG TTG GAG ATT CTT CTG CCC TTG GAA GAT CTG GAG GCT CGT GTC AAT GCT GCT GCA GAC
Gln Gln Phe Thr Gly Ile Glu Tyr Asn Lys Leu Leu Glu Ile Leu Leu Pro Leu Glu Asp Leu Glu Ala Arg Val Asn Ala Ala Ala Asp
                  1998                2016                2034                2052                2070
CTT ATA ACT CAG ATT CAT AAA AAC ATC AAC CGT GAA GCA CTC AGC TTT GCT GCT GCT TCC TTT TAC CAT AAG CTG AAG GCT GCT GAC AAG
Leu Ile Thr Gln Ile His Lys Asn Ile Asn Arg Glu Ala Leu Ser Phe Ala Ala Ala Ser Phe Tyr His Lys Leu Lys Ala Ala Asp Lys
                  2088                2106                2124                2142                2160
TAT ATA CCA GAA TCC AAG TAT CAT GGG AAC GTG ACA CTG ATG CGG GCA AAG ACT CAC AAT GAG TAT GAA GAA GGT CTG GGT GGA GAC TAC
Tyr Ile Pro Glu Ser Lys Tyr His Gly Asn Val Thr Leu MET Arg Ala Lys Thr His Asn Glu Tyr Glu Glu Gly Leu Gly Gly Asp Tyr
                  2178                2196                2214                2232                2250
AGA CTC TCA GAG GTC TGC GAT GGA AAA GTA TCA GTC CAC ATC ATT GAA GGA GAT CAC CGC ACC TTA TTG GAG GGA GAT GGT GTT GAA TCA
Arg Leu Ser Glu Val Cys Asp Gly Lys Val Ser Val His Ile Ile Glu Gly Asp His Arg Thr Leu Leu Glu Gly Asp Gly Val Glu Ser
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FIG. 2. (*Figure continues on the opposite page.*)

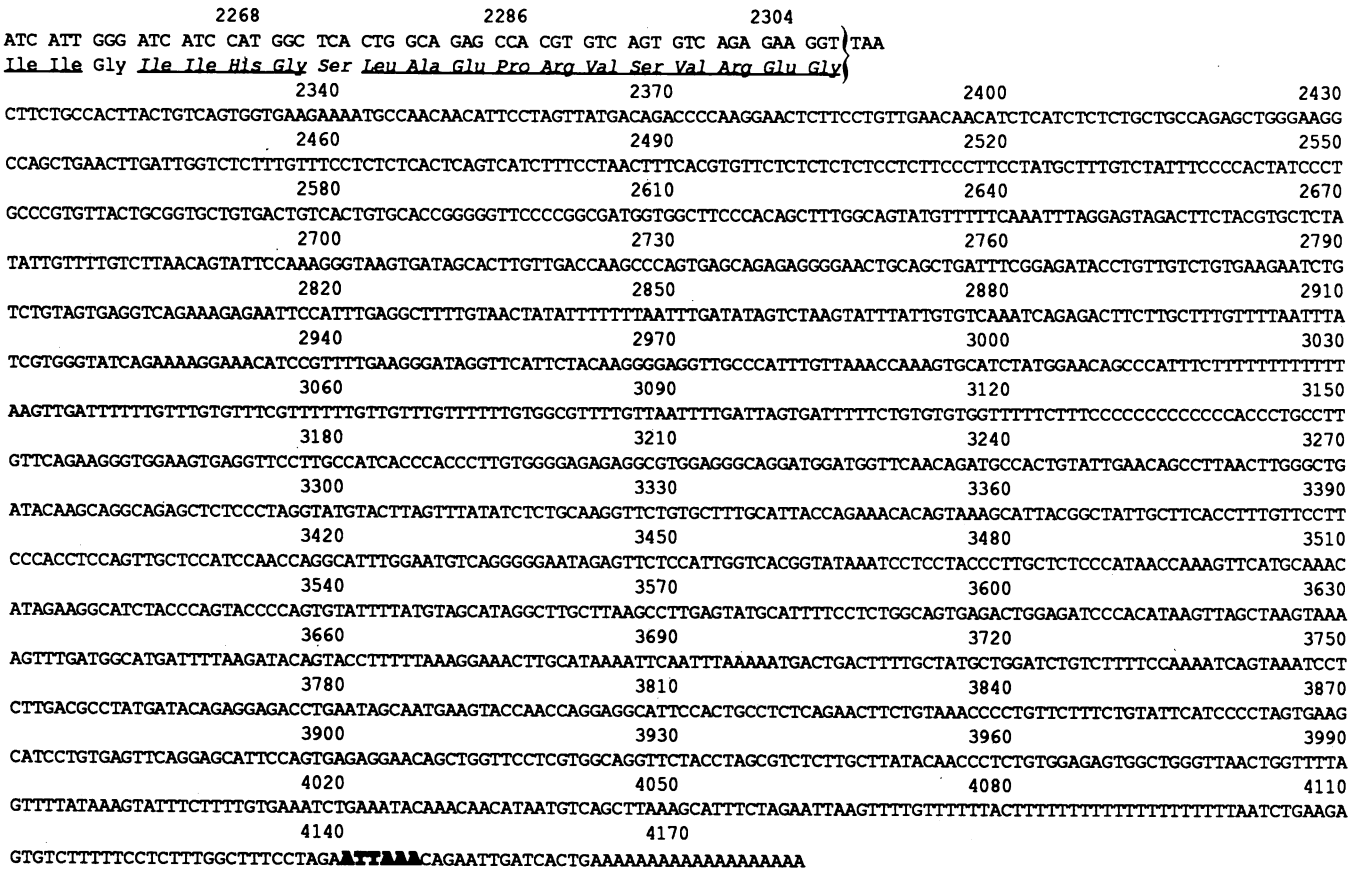

FIG. 2. cDNA sequence of clone 3 and corresponding amino acid sequence. The sequences in the two sets of parentheses correspond to the acyl carrier protein (positions 1108–1371) and thioesterase (positions 1872–2307). The boxed sequence is the 24 extra nucleotides absent in clone 10. Underlined amino acid sequences represent matches with the rat fatty acid synthase (10). The polyadenylylation signal ATTAAA is indicated in boldface type.

other, different organisms apparently used different amino acid sequences in the connecting region. Alternatively, a single joining event might have been susceptible to extensive mutation since the connections are not essential for enzymatic function. More amino acid sequence information is needed to evaluate these hypotheses.

The 24 nucleotides from 1798 to 1821 exist in clone 3 but not in clone 10. The data of Naggert *et al.* (10) and Ranganna *et al.* (9) also do not contain these extra nucleotides. Two explanations exist: error during cDNA synthesis or the existence of two different fatty acid synthase mRNAs. There is no stop codon within these 24 extra nucleotides. The position where the extra nucleotides are inserted is just at the end of the codon for a glutamic acid, and 24 extra nucleotides will insert 8 amino acids without shifting the reading frame. These facts make the possibility of error during cDNA synthesis unlikely. Previous studies (7, 8, 17) suggest that two different molecular weight mRNA species may exist because of the different lengths of the 3′ noncoding region. It is possible that one mRNA has a different number of nucleotides not only in the 3′ noncoding region, but also in the reading frame.

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