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Structure of the coding sequence and primary amino acid sequence of acetyl-coenzyme A carboxylase

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ABSTRACT **Acetyl-coenzyme A carboxylase (Ac-CoA carboxylase; EC 6.4.1.2) catalyzes the rate-limiting reaction in long-chain fatty acid biosynthesis. To investigate the mechanism of genetic control of expression of Ac-CoA carboxylase and the relationship between its structure and function, cDNA clones for Ac-CoA carboxylase were isolated. The complete coding sequence contains 7035 bases; it encodes a polypeptide chain of 2345 amino acids having a M_r of 265,220. The sequences of several CNBr peptides of Ac-CoA carboxylase were localized within the predicted protein sequence as were those peptides that contain the sites for phosphorylation. The deduced protein contains one putative site for biotinylation in the NH_2-terminal half. The "conserved" biotinylation site peptide, Met-Lys-Met, is preceded by valine, whereas alanine is found in a similar position in all other known biotin-containing proteins. The primary sequences of Ac-CoA carboxylase and carbamoyl phosphate synthetase exhibit substantial identity.**

Extensive studies have been performed in order to understand the mechanisms that regulate acetyl-coenzyme A carboxylase (Ac-CoA carboxylase; EC 6.4.1.2), the rate-limiting enzyme in the biogenesis of long-chain fatty acids. Although many aspects of the short-term allosteric and covalent modification regulatory mechanisms have been elucidated (1–3), the relationship between structure and function at the molecular level remained obscure because the primary structure of the enzyme was unknown. Ac-CoA carboxylase is an unusually large enzyme; the protomer consists of two identical subunits whose M_r is about 260,000 (4, 5). The correspondingly large size of the mRNA, about 10 kilobases (kb), and the small amount of mRNA in the cells (6) have presented some formidable experimental difficulties in bringing the molecular biology approach to bear on the problem of Ac-CoA carboxylase.

Recently, we have been able to isolate several cDNA clones from a λgt11 expression library directing the synthesis of a recombinant protein that reacts with anti-Ac-CoA carboxylase serum (7). We have now isolated and sequenced cDNA clones corresponding to the entire Ac-CoA carboxylase coding region.

In this communication, we present the nucleotide sequence† and discuss some characteristics of the deduced primary amino acid sequence of Ac-CoA carboxylase.

MATERIALS AND METHODS

Materials. The Klenow fragment of *Escherichia coli* DNA polymerase I was from Boehringer Mannheim. T4 DNA polymerase, terminal transferase, and T4 DNA ligase were from International Biotechnologies (New Haven, CT). The nick-translation kit was from Amersham. Restriction endonucleases were from Bethesda Research Laboratories. ^{32}P-labeled nucleotides were from ICN. All other chemicals were analytical reagent grade. Oligonucleotides were synthesized in an Applied Biosystems (Foster City, CA) 380A DNA synthesizer.

Isolation of Clones and Labeling Probes. All cDNA clones have been isolated from the λgt11 library whose construction has been described (7). Initially, the 5′ and 3′ ends of λKHR18 and λDH3 were used to obtain overlapping clones. Probe DNA fragments were labeled with ^{32}P by nick-translation using a commercial kit (8). Immobilization of phage DNA on nitrocellulose paper and hybridization conditions were the same as described by Berent *et al.* (9).

DNA Sequencing. DNA inserts were routinely subcloned into pUC19 and then subcloned into M13mp19 by using standard techniques (10). DNA was sequenced in both directions by the dideoxy chain-termination method (11) using the original M13 subclones and their deletion derivatives generated according to Dale *et al.* (12). In some cases it was necessary to employ specific oligonucleotides as primers in the Sanger's reaction (13) or to use the Maxam–Gilbert chemical cleavage method of DNA sequencing (14).

Ac-CoA Carboxylase Gene Copy Number Determination. Gene copy number was determined by the method of Back *et al.* (15). High molecular weight genomic DNA was isolated from rat mammary glands as described by Nathans and Hogness (16), digested with high concentrations of restriction endonucleases, and subjected to Southern blot analysis (10) using ^{32}P-labeled riboprobes. Digestion of the genomic DNA with *Eco*RI gave rise to a 3.0-kilobase-pair (kbp) DNA fragment that is recognized by the first 194 bases of clone λDHN-132. This unique hybridization signal was used to determine the gene copy number per haploid chromosome set. The amount of DNA that was used in the Southern blot analysis was quantitated by using 33258-Hoechst fluorochrome as described by Cesarone *et al.* (17). The DNA that was used for the construction of the standard curve for gene copy titration was an extensively purified pGEM3 plasmid subclone (pCX321) that releases a 3.3-kbp cDNA fragment upon *Eco*RI digestion. This cDNA contains one copy of the 194 bp of the 5′ end of λDHN-132. Two picograms of pCX321 and 1.35 mg of rat genomic DNA should produce equivalent signals upon hybridization if the number of Ac-CoA carboxylase gene copies per haploid chromosome set is one (18). After hybridization and exposure, films were analyzed by using an LKB automated densitometer.

RESULTS AND DISCUSSION

Coding and Deduced Amino Acid Sequences of Ac-CoA Carboxylase. The initial screening of the λgt11 library with

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Abbreviation: Ac-CoA, acetyl-coenzyme A.

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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. J03808).

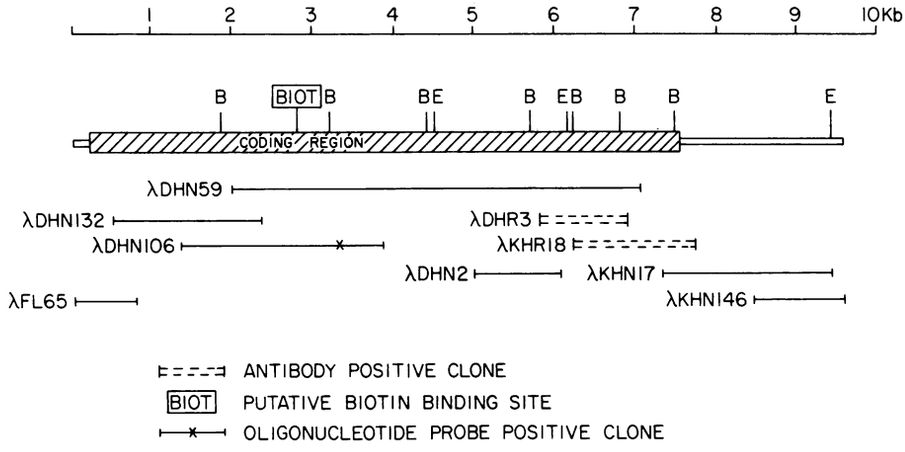

FIG. 1. Summary diagram of cDNA clones. Representative clones whose sequences were determined are shown with *Bam*HI (B) and *Eco*RI (E) restriction sites.

anti-Ac-CoA carboxylase serum identified several clones that contain sequences for Ac-CoA carboxylase. Two of these positive clones, λDHR3 and λKHR18, have been extensively characterized (7). To isolate additional overlapping clones, cDNA fragments obtained from λDHR3 and λKHR18 were initially utilized as probes for screening the λgt11 library and then subsequently identified clones were similarly used.

Fig. 1 shows how the various clones cover the entire coding sequence of Ac-CoA carboxylase mRNA. The restriction map of these clones is shown in Fig. 1. Whenever there were any *Eco*RI sites in the cDNA insert, such as λDHR3, the nucleotide sequence around them was confirmed by sequencing the M13 subclones that were obtained by using restriction enzymes other than *Eco*RI. This procedure prevented the omission of small fragments that might have occurred between two internal *Eco*RI sites. Analysis of sequence data from all of the clones established only one open reading frame potentially large enough to code for Ac-CoA carboxylase; it is flanked by several in-frame stop codons. Translation from the first available methionine in this open reading frame defines the coding region of Ac-CoA carboxylase mRNA, which consists of 7038 bases including the termination signal, TAG (Fig. 2). This region encodes a protein of 2345 amino acids with a M_r of 265,220. The amino acid composition (Table 1) of the deduced protein is in good agreement with that obtained from the purified enzyme from rat liver (22). Previously, the M_r of Ac-CoA carboxylase from rat liver (4) and mammary gland (5) was reported to be about 260,000.

Due to the size of the open reading frame for Ac-CoA carboxylase, it was desirable to confirm our sequence data by independent additional experimental techniques. Four peptides from CNBr digests of rat liver Ac-CoA carboxylase were isolated by high-performance liquid chromatography. NH_2-terminal amino acid sequences were determined for each peptide and were compared to the amino acid sequence deduced from our overlapping cDNA clones. As shown in Fig. 2, the amino acid sequence of peptide Y-20 was found to be identical to the deduced sequence located between residues 628 and 651; similarly, peptide Y-15 corresponds to residues 921–938, peptide Y-12 corresponds to residues 1306–1318, and peptide Y-4 corresponds to residues 1570–1581, as indicated. In addition, four peptide sequences that contain seven phosphorylation sites of Ac-CoA carboxylase (19–21) are also found in this deduced amino acid sequence (Fig. 2). Between the 5′ untranslated sequence, which contains several in-frame stop codons and the phosphopeptide T4, there is one in-frame codon for methionine. Therefore, this ATG must be the translation initiation codon; it is shown as such in Fig. 2.

An important feature of every biotin-containing enzyme is the presence of a canonical biotin binding site sequence, Met-Bct-Met, in which Bct represents the biotin prosthetic group covalently attached to the ε-NH_2 group of a lysine residue in the polypeptide chain (23). Only one such sequence occurring at residues 784–786 was found in our predicted Ac-CoA carboxylase sequence (Fig. 2). Comparison of the amino acid sequence around this putative biotin binding site with those reported for other biotin-containing enzymes (23) indicates that this may be the biotin binding site for rat Ac-CoA carboxylase. Unequivocal proof that the lysine in the Val-Met-Lys-Met sequence represents the biotin binding site requires additional investigation.

One of the interesting findings in the deduced Ac-CoA carboxylase amino acid sequence is a strong homology with yeast (24) and rat carbamoyl phosphate synthetase (25). Analysis using the FASTP program (26) indicates that Ac-CoA carboxylase (residues 273–469) and yeast carbamoyl phosphate synthetase (residues 152–349) show statistically

Table 1. Ac-CoA carboxylase amino acid composition

Amino acid	% of amino acid per mol of enzyme 1*	2†
Ala	6.5	6.5
Arg	6.1	6.0
Asn	4.5	—
Asp	5.5	9.2
Cys	1.2	1.5
Gln	4.4	—
Glu	6.9	11.9
Gly	6.1	6.2
His	2.6	2.9
Ile	6.1	5.5
Leu	9.2	9.1
Lys	4.8	5.0
Met	3.2	3.1
Phe	4.1	4.1
Pro	4.6	4.9
Ser	6.6	6.1
Thr	4.6	4.9
Trp	1.2	1.5
Tyr	3.4	3.4
Val	8.2	8.1

*Predicted composition from cDNA sequence of Ac-CoA carboxylase from rat mammary gland.
†Rat liver Ac-CoA carboxylase (22).

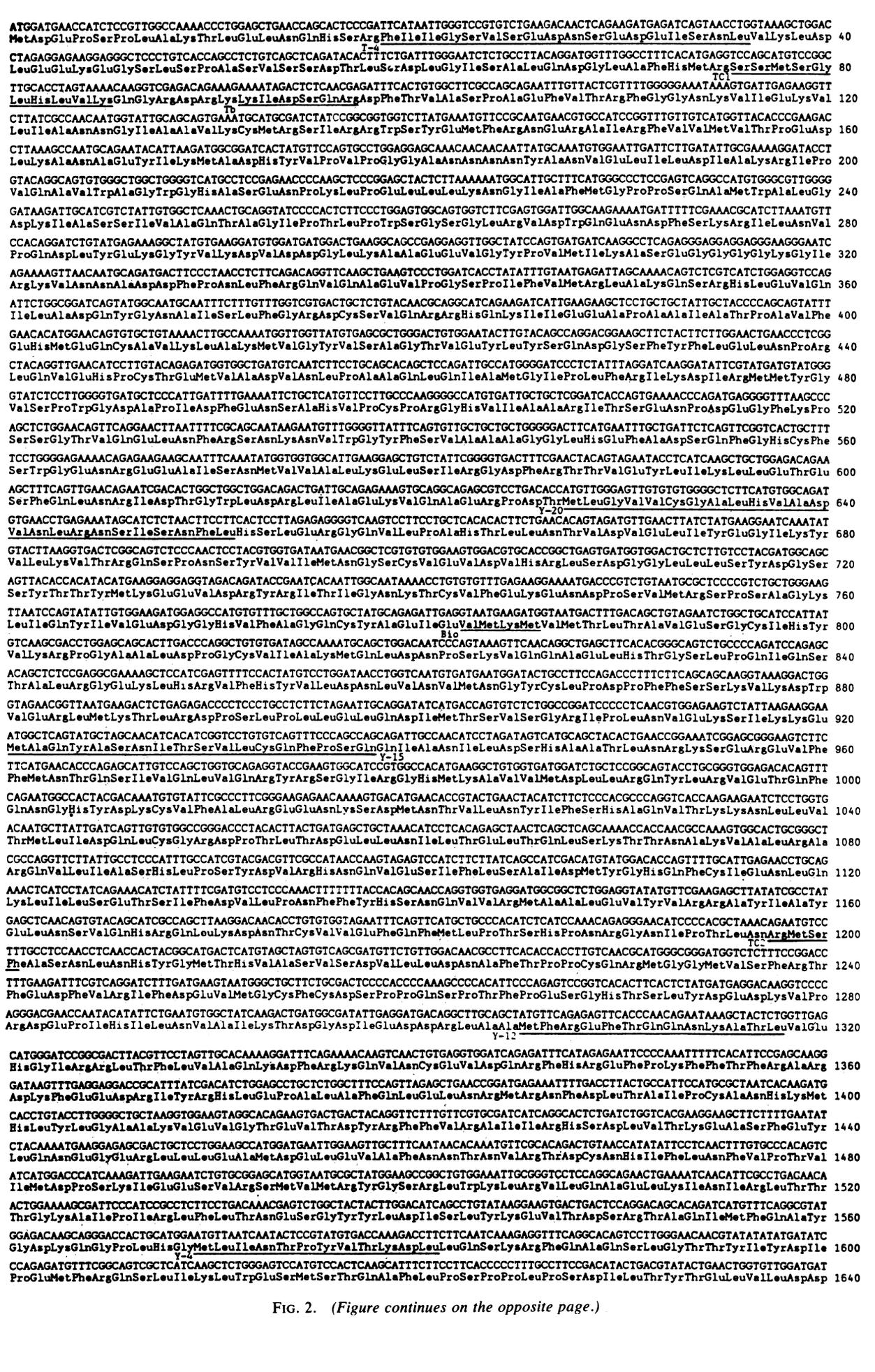

FIG. 2. *(Figure continues on the opposite page.)*

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CAAGGCCAGCTTGTCCATATGAACAGACTTCCAGGAGGAAACGAGATTGGCATGGTAGCCTGGAAAATGAGCCTTAAAAGCCCTGAATATCCAGATGGCCGAGATGTCATTGTCATCGGC
GlnGlyGlnLeuValHisMetAsnArgLeuProGlyGlyAsnGluIleGlyMetValAlaTrpLysMetSerLeuLysSerProGluTyrProAspGlyArgAspValIleValIleGly 1680

AATGACATTACATATCGGATTGGCTCCTTTGGGCCTCAGGAAGATTTGCTGTTTCTCAGAGCTTCTGAACTTGCCAGAGCAGAAGGCATCCCACGCATCTACGTAGCAGCCAACAGTGGA
AsnAspIleThrTyrArgIleGlySerPheGlyProGlnGluAspLeuLeuPheLeuArgAlaSerGluLeuAlaArgAlaGluGlyIleProArgIleTyrValAlaAlaAsnSerGly 1720

GCTAGAATTGGACTGGCAGAAGAAATCCGTCATATGTTCCACGTGGCCTGGGTAGACTCTGAGGATCCTTACAAGGGATACAAGTATTTATATCTGACACCCCAGGATTATAAAAGAGTG
AlaArgIleGlyLeuAlaGluGluIleArgHisMetPheHisValAlaTrpValAspSerGluAspProTyrLysGlyTyrLysTyrLeuTyrLeuThrProGlnAspTyrLysArgVal 1760

AGTGCTCTCAATTCTGTCCACTGTGAACATGTGGAAGATGAAGGAGAATCCAGGTACAAGATAACAGACATTATCGGGAAAGAAGAAGGACTTGGAGCAGAGAACCTTCGGGGTTCTGGA
SerAlaLeuAsnSerValHisCysGluHisValGluAspGluGlyGluSerArgTyrLysIleThrAspIleIleGlyLysGluGluGlyLeuGlyAlaGluAsnLeuArgGlySerGly 1800

ATGATTGCTGGGGAATCCTCATTGGCTTATGATGAGATCATCACCATCAGCCTGGTTACATGCCGGGCCATTGGTATTGGGGCTTACCTTGTCCGGCTGGGACAAAGAACCATCCAGGTT
MetIleAlaGlyGluSerSerLeuAlaTyrAspGluIleIleThrIleSerLeuValThrCysArgAlaIleGlyIleGlyAlaTyrLeuValArgLeuGlyGlnArgThrIleGlnVal 1840

GAGAACTCTCACTTAATTCTAACAGGAGCCGGTGCCCTCAACAAAGTCCTTGGTCGGGAAGTATACACCTCCAACAATCAGCTTGGGGGCATCCAGATAATGCACAACAACGGAGTTACC
GluAsnSerHisLeuIleLeuThrGlyAlaGlyAlaLeuAsnLysValLeuGlyArgGluValTyrThrSerAsnAsnGlnLeuGlyGlyIleGlnIleMetHisAsnAsnGlyValThr 1880

CATTGCACTGTTTGTGATGACTTTGAGGGAGTGTTCACAGTCTTACACTGGCTGTCATACATGCCTAAGAACGTGCACAGTTCAGTTCCTCTCCTGAATTCCAAGGATCCTATAGATAGA
HisCysThrValCysAspAspPheGluGlyValPheThrValLeuHisTrpLeuSerTyrMetProLysAsnValHisSerSerValProLeuLeuAsnSerLysAspProIleAspArg 1920

ATCATCGAGTTTGTTCCCACAAAGGCCCCGTATGATCCTCGGTGGATGCTGGCAGGCCGTCCTCACCCAACCCAGAAAGGCCAATGGTTGAGTGGATTTTTTGATTATGGCTCTTTCTCA
IleIleGluPheValProThrLysAlaProTyrAspProArgTrpMetLeuAlaGlyArgProHisProThrGlnLysGlyGlnTrpLeuSerGlyPhePheAspTyrGlySerPheSer 1960

GAAATCATGCAGCCCTGGGCGCAGACCGTGGTAGTTGGCAGAGCCAGGTTGGGGGGAATACCTGTGGGAGTAGTTGCTGTAGAAACCCGAACCGTGGAGCTCAGTGTACCAGCTGATCCT
GluIleMetGlnProTrpAlaGlnThrValValValGlyArgAlaArgLeuGlyGlyIleProValGlyValValAlaValGluThrArgThrValGluLeuSerValProAlaAspPro 2000

GCAAACCTGGATTCTGAAGCCAAGATAATCCAGCAGGCCGGCCAAGTTTGGTTTCCAGACTCTGCATTTAAGACCTATCAAGCTATCAAGGACTTTAACCGTGAAGGGCTACCTCTAATG
AlaAsnLeuAspSerGluAlaLysIleIleGlnGlnAlaGlyGlnValTrpPheProAspSerAlaPheLysThrTyrGlnAlaIleLysAspPheAsnArgGluGlyLeuProLeuMet 2040

GTCTTTGCCAACTGGAGAGGCTTCTCTGGTGGGATGAAAGATATGTATGACCAGGTGCTCAAGTTTGGTGCTTATATTGTGGATGGCTTGCGGGAATGTTCCCAGCCTGTGATGGTCTAC
ValPheAlaAsnTrpArgGlyPheSerGlyGlyMetLysAspMetTyrAspGlnValLeuLysPheGlyAlaTyrIleValAspGlyLeuArgGluCysSerGlnProValMetValTyr 2080

ATTCCCCCACAGGCTGAGCTTCGGGGTGGTTCTTGGGTTGTGATCGACCCAACCATCAATCCTCGGCACATGGAGATGTATGCTGACCGGGAAAGCAGGGGATCCGTTCTGGAACCAGAA
IleProProGlnAlaGluLeuArgGlyGlySerTrpValValIleAspProThrIleAsnProArgHisMetGluMetTyrAlaAspArgGluSerArgGlySerValLeuGluProGlu 2120

GGGACAGTAGAAATCAAATTCCGCAAAAAGGATCTGGTGAAAACCATGCGTCGCGTAGACCCAGTCTACATCCGCTTGGCTGAGCGACTGGGCACCCCAGAGCTAAGCCCCACTGAGCGG
GlyThrValGluIleLysPheArgLysLysAspLeuValLysThrMetArgArgValAspProValTyrIleArgLeuAlaGluArgLeuGlyThrProGluLeuSerProThrGluArg 2160

AAGGAGCTGGAGAGCAAGTTGAAGGAGCGGGAGGAGTTCCTAATTCCCATTTACCATCAGGTAGCTGTGCAGTTTGCTGACTTGCACGACACCCCAGGCCGGATGCAGGAGAAGGGTGTC
LysGluLeuGluSerLysLeuLysGluArgGluGluPheLeuIleProIleTyrHisGlnValAlaValGlnPheAlaAspLeuHisAspThrProGlyArgMetGlnGluLysGlyVal 2200

ATTAATGATATCTTAGATTGGAAAACATCCCGCACCTTCTTCTACTGGCGACTGAGGCGTCTCCTGCTGGAAGACCTGGTCAAGAAGAAAATCCACAGTGCCAACCCTGAGCTGACCGAT
IleAsnAspIleLeuAspTrpLysThrSerArgThrPhePheTyrTrpArgLeuArgArgLeuLeuLeuGluAspLeuValLysLysLysIleHisSerAlaAsnProGluLeuThrAsp 2240

GGCCAGATCCAGGCCATGTTGAGACGCTGGTTTGTGGAAGTGGAAGGCACAGTGAAGGCTTACGTCTGGGACAATAATAAGGATCTGGTGGAATGGCTGGAGAAGCAGCTGACAGAGGAA
GlyGlnIleGlnAlaMetLeuArgArgTrpPheValGluValGluGlyThrValLysAlaTyrValTrpAspAsnAsnLysAspLeuValGluTrpLeuGluLysGlnLeuThrGluGlu 2280

GATGGTGTCCGCTCTGTGATAGAGGAGAACATCAAATACATCAGCAGGGACTATGTCCTCAAGCAGATCCGCAGCTTGGTGCAGGCCAATCCAGAAGTTGCCATGGACTCCATCGTCCAC
AspGlyValArgSerValIleGluGluAsnIleLysTyrIleSerArgAspTyrValLeuLysGlnIleArgSerLeuValGlnAlaAsnProGluValAlaMetAspSerIleValHis 2320

ATGACCCAGCACATCTCCCCCACTCAGCGAGCAGAGGTTGTAAGGATCCTTTCCACTATGGACTCCCCTTCTACGTAG
MetThrGlnHisIleSerProThrGlnArgAlaGluValValArgIleLeuSerThrMetAspSerProSerThrEnd 2346
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FIG. 2. Complete coding sequence for Ac-CoA carboxylase. All clones were sequenced on both strands. Positions of the amino acid sequences of the peptides isolated from the purified Ac-CoA carboxylase as well as the putative biotin binding site are underlined. T4, Tb, TC1, and TC2 represent phosphopeptides whose sequences were determined (19–21); Bio, biotin binding site; Y-12, Y-15, and Y-20, purified peptides from CNBr digest of Ac-CoA carboxylase.

significant homology (Z_i = 9.39 and Z_{opt} = 24.66). Carbamoyl phosphate synthetase catalyzes the reaction

$$NH_4^+ + HCO_3^- + ATP \rightleftarrows H_2N{-}\overset{\overset{O}{\|}}{C}{-}O{-}PO_3^{2-} + ADP,$$

whereas Ac-CoA carboxylase, in the absence of Ac-CoA, catalyzes the half-reaction

$$\text{E-biotin} + HCO_3^- + ATP \rightleftarrows \text{E-biotin}{-}\overset{\overset{O}{\|}}{C}{-}O^- + ADP + P_i$$

(where E = enzyme). Thus, it is reasonable to postulate that the homologous regions of these enzymes are involved in binding HCO_3^- and ATP. Previously, chicken Ac-CoA carboxylase and the biotin carboxylase component of *E. coli* Ac-CoA carboxylase (2) were shown to catalyze the following reaction in the presence of biotin:

$$H_2N{-}\overset{\overset{O}{\|}}{C}{-}O{-}PO_3^{2-} + ADP \rightleftarrows H_2N{-}\overset{\overset{O}{\|}}{C}{-}O^- + ATP.$$

This reaction was used as a model for studying the mechanism of reaction of biotin in Ac-CoA carboxylase (27).

The α-subunit of propionyl-CoA carboxylase is the subunit to which biotin binds (28). At least in the bacterial enzyme, it has been shown that the α-subunit alone can carry out the carboxylation reaction but not the overall reaction unless the β-subunit is added, indicating that the β-subunit may contain the acyl-CoA binding site (29). A comparison between the primary amino acid sequence of Ac-CoA carboxylase and the β-subunit of human propionyl-CoA carboxylase (28) through the FASTP program identified some homology between Ac-CoA carboxylase (residues 1959–1990) and the β-subunit (residues 40–70). When this region of the amino acid sequence was compared to the known "adenosine recognition loop" of citrate synthetase (30–31), a possible CoA binding site could be identified, as shown in Fig. 3. However, at this time we have no experimental proof that this region is the adenosine recognition loop.

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Rat ACC (1958-1987)   S F S E I M Q P W A Q T V V V G R A R L G G I P V G V V A V
PC-β rat (330-359)    E F F E I M P N Y A K N I V I G F A R M N G R T V G I V G N
PC-β human (40-70)    E F F E I M P N Y A K N I I V G F A R M N G R T V G I V G N
PCS (309-338)         R D Y I W N T L N S G R V V P G Y G H A V L R K T D P R Y T
YCS (309-338)         E K Y L W D T L N A G R V V P G Y G H A V L R K T D P R Y T
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FIG. 3. Alignment of homologous sequences among CoA binding proteins. Numbers in parentheses indicate the amino acid starting position in each protein. PCS, porcine citrate synthetase (31); YCS, yeast citrate synthetase (31); PC-β rat, propionyl-CoA carboxylase β-subunit (32); PC-β human, the β-subunit of human propionyl-CoA carboxylase (28); ACC, Ac-CoA carboxylase.

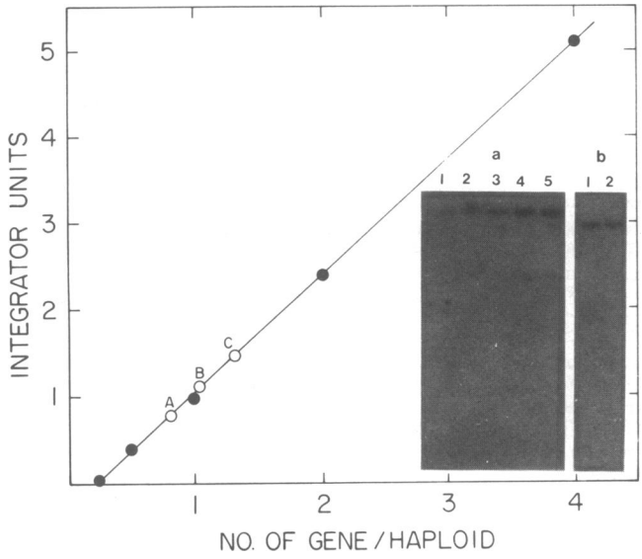

FIG. 4. Titration of gene copy number. Plasmid pCX321 and genomic DNA from rat liver were digested with *Eco*RI. After digestion, different amounts of DNA were separated by 1% agarose gel electrophoresis and subjected to Southern blot analysis (10). The *Inset* shows 0.7 pg (*a*, lane 1), 1.4 pg (*a*, lane 2), 2.8 pg (*a*, lane 3), 5.6 pg (*a*, lane 4), and 11.2 pg (*a*, lane 5) of pCX321 and 1.35 μg of digested genomic DNA from two animals (*b*, lanes 1 and 2). The open circles labeled A and B correspond to *b*, lanes 1 and 2, respectively. The value for the open circle labeled C was obtained in a separate experiment. The intensity of the hybridization signal was measured against the background of a lane without any DNA.

Ac-CoA Carboxylase Gene Copy Number. Sequencing a cDNA as large as that for Ac-CoA carboxylase requires identification of many clones whose sequences overlap so that the overall sequence can be constructed. The analysis of newly detected clones would be greatly simplified if the protein under study had only one gene copy per haploid chromosome set. To determine the gene copy number, we established a unique restriction pattern for the Ac-CoA carboxylase-encoding region of rat genomic DNA. The *Eco*RI digest of high molecular weight genomic DNA was analyzed by using the first 194 bases to the 5′ end of λDHN-132 in Southern blots of the genomic DNA. Only one restriction fragment, 3 kb, was hybridized (Fig. 4 *Inset b*). Similar results were obtained with *Alu* I and *Hin*dIII (results not shown). The uniqueness of fragment recognition with such a small probe for three different restriction enzymes strongly suggests the existence of only one copy of the probed gene per haploid chromosome set.

To provide further verification that there is only one Ac-CoA carboxylase gene copy per haploid chromosome set, we titrated the 3.0-kbp *Eco*RI genomic fragment by using the first 194 bases of λDHN-132 as a probe. We have compared the intensity of the hybridization signals for Ac-CoA carboxylase in genomic DNA digested with *Eco*RI to that of a known standard. The averaging of three independent determinations (A–C in Fig. 4) gave a value of 1.17 copies per haploid chromosome set. These studies indicate that there is one Ac-CoA carboxylase gene per haploid chromosome set in the rat liver genome.

In summary, we have determined the primary amino acid sequence of rat Ac-CoA carboxylase as deduced from the nucleotide sequence of cDNA clones encompassing the entire coding region of Ac-CoA carboxylase mRNA. Such an amino acid sequence correctly predicts the known molecular weight of the enzyme protomer. There is exact agreement between the deduced amino acid sequence and several isolated CNBr peptides of Ac-CoA carboxylase. In addition, there is good agreement between the determined and deduced amino acid compositions. The primary amino acid sequence appears to contain one putative biotin binding site.

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