Heterogeneity at the 5' End of Rat Acetyl-coenzyme A Carboxylase mRNA

LIPOGENIC CONDITIONS ENHANCE SYNTHESIS OF A UNIQUE mRNA IN LIVER*

Fernando López-Casillas and Ki-Han Kim*

From the Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907

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Multiple forms of acetyl-CoA carboxylase mRNA were previously detected in the mammary gland (López-Casillas, F., Luo, X., Kong, I.-S., and Kim, K.-H. (1989) Gene, in press). We have now established that the rat liver also contains heterogeneous acetyl-CoA carboxylase mRNA populations that differ in the 5'-untranslated region. In addition, the liver contains a unique form of acetyl-CoA carboxylase mRNA in which the 5'-nontranslated end differs from the species in mammary gland. The 5' end of this unique species was characterized using a procedure for cloning minute amounts of primer extension products (pAU clones). This procedure should also be useful for obtaining full length clones of other mRNAs.

The DNA sequence of pAU clones indicates that this liver-specific acetyl-CoA carboxylase mRNA has a 315-base long untranslated region. The first 242 nucleotides replace the 5' end of the predominant acetyl-CoA carboxylase mRNA found in the mammary gland (FL56 type). Under lipogenic conditions the unique liver acetyl-CoA carboxylase mRNA increases and is the major species of acetyl-CoA carboxylase mRNA. Livers from rats fed a normal diet and the mammary glands of lactating rats do not contain detectable amounts of the pAU type mRNA. On the other hand, the epididymal adipose tissue from these animals contains mainly the pAU type and only minimal amounts of the FL56 type. The multiple forms of acetyl-CoA carboxylase mRNA appear to be generated by differential splicing. In addition, transcription appears to be physiologically regulated by the use of tissue-specific acetyl-CoA carboxylase gene promoters.

Acetyl-CoA carboxylase catalyzes the rate-limiting reaction in the biosynthesis of long chain fatty acids: the ATP-dependent carboxylation of acetyl-CoA into malonyl-CoA (1,2). To elucidate the control mechanisms that regulate acetyl-CoA carboxylase at the gene level, we have recently purified acetyl-CoA carboxylase mRNA from lactating rat mammary gland (3), cloned its cDNA (4), and sequenced its coding region (5). Acetyl-CoA carboxylase mRNA is encoded in a single gene copy per haploid chromosome set (5). The amount of acetyl-CoA carboxylase mRNA is very small even during lactation, a condition that demands a high rate of fatty acid synthesis (3). Acetyl-CoA carboxylase mRNA with an apparent size of 10 kilobases (3, 4) contains an open reading frame of 7,035 nucleotides that encodes a 265,220-dalton polypeptide of 2,345 amino acids (5).

The lactating rat mammary gland contains multiple forms of acetyl-CoA carboxylase mRNA that differ in the 5'-nontranslated end. This tissue produces two types of acetyl-CoA carboxylase mRNA that differ solely with respect to the presence (FL63) or absence (FL56) of a 61-base insertion in the middle of the 5'-untranslated end. The regions upstream of the 61-base insert have a high (C + G) content, a high frequency of the CpG dinucleotide, and originate from the first exon(s) in the acetyl-CoA carboxylase transcriptional unit. While characterizing the 5' end of acetyl-CoA carboxylase mRNA, we found a novel form of rat liver acetyl-CoA carboxylase mRNA that is not detected in the mammary gland. We herein describe the 5' end of hepatic acetyl-CoA carboxylase mRNA (the pAU type), which becomes the major form of acetyl-CoA carboxylase mRNA under lipogenic conditions. This novel acetyl-CoA carboxylase mRNA is also present in the epididymal adipose tissue of rats given a normal diet. This pAU type of acetyl-CoA carboxylase mRNA contains a different 242-nucleotide sequence in place of the (C + G)-rich region of the FL56 type. Our data suggest the existence of an alternative promoter in the acetyl-CoA carboxylase gene, which is active in liver and adipose tissue but inactive in the mammary gland.

EXPERIMENTAL PROCEDURES

Materials—Wistar rats were from our departmental animal colonies. Enzymes and chemicals were purchased from the following suppliers: T4 DNA polynucleotide kinase and fat-free diet from United States Biochemical Corp.; DNA polymerase Klenow fragment, calf intestinal alkaline phosphatase, and dideoxyribonucleoside triphosphates (ddNTPs)² from Boehringer Mannheim; terminal deoxynucleotide transferase, T4 DNA ligase, EcoRI, TaqI, BamHI, EcoRV, Smal, and HindIII restriction endonucleases from Bethesda Research Laboratories; S1 nuclease and deoxyribonucleoside triphosphates (dNTPs) from Pharmacia LKB Biotechnology Inc.; avian myeloblastosis virus (AMV) reverse transcriptase from Life Sciences Inc.; T4 DNA polymerase from Promega Biotech; [α-32P]ATP (600 Ci/mmole) from Du Pont-New England Nuclear; α-32S-dATP (600 Ci/mmole) from Amersham Corp.; and guanidinium thiocyanate from Fluka.

²The abbreviations used are: ddNTP, dideoxyribonucleoside triphosphates; dNTP, deoxyribonucleoside triphosphates; AMV, avian myeloblastosis virus; DTT, dithiothreitol; Pipes, 1,4-piperazinediethanesulfonic acid.

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To whom correspondence should be addressed.

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other chemicals were reagent grade.

RNA Preparations—Total RNA was prepared from the mammary glands of lactating rats on the 5th-7th day postpartum, using a slight modification (5) of the protocol of Cathala et al. (6). The guanidinium thiocyanate method described by MacDonald et al. (7) was employed to prepare RNA from livers and mammary gland fat pads.

Primers, Probes, and Extended Primer Synthesis—Oligonucleotides 218 5'ATGTTTCATCAGATTATC-3' and 263 5'-TGATCTCATCTTTGTTCTGACGTCGACGTTAAATT-3' were synthesized in an Applied Biosystems 380A DNA synthesizer and purified by phenol/chloroform/glycol electrophoresis (8). The synthesis of primer 263/H2/Taq 108 nucleotides long was used to be described the strategy employed in the synthesis of the "extended" 32P-oligonucleotides (primers and probes) utilized in this paper. The primers were named to reflect the way they were synthesized. Oligorotinoctotide 263 was labeled at its 5' end by using [32P]ATP and T4 polynucleotide kinase as described by Taylor et al. (9). An aliquot of the kinase reaction mixture containing ~30 pmol of the 32P-oligorotinoctotide (primers and probes) utilized in this paper. The primers were resuspended in 150 µl of DEP-treated H2O and stored at −70°C.

Primer Extension and RNA Sequencing—Primer extension was performed as described by Berthod et al. (12). Briefly, 100-200 µg of extended primer (-15,000-30,000 cpm) was hybridized with 100-200 µg of total RNA in 50 µl of 0.14 M Pipes, pH 6.4, containing 80% formamide, 0.4 M NaCl, and 1 mM EDTA by heating to 70°C for 15 min and then incubating at 43°C for 4.5 h. Annealed primer/rRNA mixture was rapidly precipitated with ethanol and resuspended in 40 µl of 50 mM Tris-HCl, pH 8.3 (measured at 42°C), containing 50 µg/ml of RNAse, 0.4 mM each of dATP, dCTP, dTTP, dGTP, dTTTP, and 25-35 units of AMV reverse transcriptase. The reaction mixture was incubated at 40°C for 60 min. The reaction was stopped by adding 60 µl of 0.3 M NaOH and incubated for an additional 60 min at 40°C. Following neutralization, the mixture was extracted with phenol/chloroform as described by Maniatis et al. (16). Ligation of cloning vector and double-stranded primer extension product cDNA was carried out in 20 µl of 70 mM Tris-HCl, pH 8.0, containing 10 mg/ml of oligo(dT) (16-20-mer) and 150 mM KCl. This mixture was heated to 65°C for 10 min, slowly cooled to room temperature, and then adjusted to 100 mM KCl, 30 mM Tris-HCl, pH 7.5, 15 mM MgCl2, 2 mM DTT, 85 µM of each dNTP, and 10 units/ml Klengow fragment in a total volume of 100 µl. The reaction mixture was incubated at 37°C for 30 min (14). The tailing reaction was stopped by the addition of 3 µl of 0.1 M EDTA, and the reaction products were purified by precipitation with ethanol and chloroform and precipitation with ethanol. To synthesize its complementary strand, the poly(A) tail primer extension product was resuspended in 30 µl of 10 mM Tris-HCl, pH 7.5, containing 100 µg/ml of oligo(dT) (16-20-mer) and 150 mM KCl. This mixture was heated to 65°C for 10 min, slowly cooled to room temperature, and then adjusted to 100 mM KCl, 30 mM Tris-HCl, pH 7.5, 15 mM MgCl2, 2 mM DTT, 85 µM of each dNTP, and 10 units/ml Klengow fragment in a total volume of 100 µl. The reaction mixture was incubated at room temperature for 45 min, extracted with phenol/chloroform, and ethanol, and the reaction products present in the aqueous phase were precipitated with ethanol. In order to generate flush ends in the double-stranded cDNAs using T4 DNA polymerase (15), the cDNAs were resuspended in 60 µl of 33 mM Tris acetate, pH 7.9, containing 68 mM potassium acetate, 10 mM magnesium acetate, 150 µM of each dNTP, and 250 units/ml T4 DNA polymerase and incubated at room temperature for 20 min. The reaction mixture was extracted with phenol and chloroform following the addition of 7 µl of 0.1 M EDTA. The double-stranded cDNA in the aqueous phase were precipitated with ethanol. Cloning vector pUC18 was prepared for ligation by digestion with 5' and dephosphorylation with calf intestinal alkaline phosphatase as described by Maniatis et al. (17). DNA sequencing—DNA inserts were subcloned into M13mp19 by using standard techniques. The sequences were determined in both directions by the dyelexone chain termination method (19).

RESULTS

Heterogeneity in the 5' End of Rat Liver Acetyl-CoA Carboxylase mRNA—Multiple products were obtained by primer extension of the 108-nucleotide fragment 263/H2/Taq (Fig. 1) with liver RNA prepared from rats that were subjected to the starvation/refeeding regimen (Fig. 2, lane 1). Two of these species are the same size as products a and b that are generated by primer extension of the predominant form of rat mammary gland acetyl-CoA carboxylase mRNA.4 However, four additional primer extension products were detected. These additional cDNAs are indicated as bands A, B, and C.
The were starved for 3 days prior to death. Under starvation the primer extension of liver RNA prepared from rats that plate used for primer extension. The partial sequence obtained to the prominent primer extension products of Figs. 2, 3B, and 8.

The cDNA species shown in Fig. 2, lane I, are the result of specific priming as indicated by the fact that they are S1 nuclease-resistant (Fig. 2, lane 2). In this experiment an aliquot of the primer extension reaction was subjected to S1 nuclease digestion. Only specific primer extension products will have full length base pairing with their RNA template and will be protected from S1 nuclease digestion.

Further proof for the specificity of the primer extension reaction was obtained by directly sequencing the RNA template used for primer extension. The partial sequence obtained from total liver RNA using primer 263/H2/Taq matches the sequence at the 5' end of the H2 exon (segment IV, Fig. 1), the 250-base long exon containing the AUG initiation codon of the acetyl-CoA carboxylase coding region (5) (Fig. 3A).

Extended exposure of the same sequencing gel allowed an extended primer extension from 263/H2/Taq (open arrow locates the unextended primer) on total liver RNA from rats subjected to starvation (3 days) and refeeding with a fat-free diet (2 days) prior to death. Prominent primer extension products are indicated as bands A, B, C, D, a, and b. Molecular weight markers (32P-labeled HpaII fragments of pBR322) are indicated in nucleotides at the margin of the figure.

FIG. 2. Primer extension (lane I) and linked primer extension/S1 nuclease digestion (lane 2) experiments performed with primer 263/H2/Taq (open arrow locates the unextended primer) on total liver RNA from rats subjected to starvation (3 days) and refeeding with a fat-free diet (2 days) prior to death. Prominent primer extension products are indicated as bands A, B, C, D, a, and b. Molecular weight markers (32P-labeled HpaII fragments of pBR322) are indicated in nucleotides at the margin of the figure.

Additional Forms of Acetyl-CoA Carboxylase mRNA—Primer extension experiments and RNA sequencing have revealed the existence of acetyl-CoA carboxylase mRNA forms in the liver that resemble the FL56 and FL63 types of acetyl-CoA carboxylase mRNA present in the mammary gland (3). In order to confirm the existence of such forms of acetyl-CoA carboxylase mRNA in the liver and to determine how the liver primer extension products A-D (Fig. 2) are structurally related to acetyl-CoA carboxylase mRNAs in the mammary gland, we carried out S1 nuclease analysis. For this experiment two 5' end-labeled cDNA specific probes were synthesized. Probe FL65/Hind (283 nucleotides) and probe FL65/Hind (360 nucleotides) can discriminate between the FL56 and FL63 types of acetyl-CoA carboxylase mRNA (Fig. 4). These probes also contain 54 bases of the M13mp19 polylinker region at their 3' ends. When these probes are used for the S1 nuclease analysis, these 54 bases will be digested from the hybrids, and the fragments that are protected by acetyl-CoA carboxylase mRNA can be unequivocally distinguished from the undigested probe. In Fig. 5 bands
f and g identify the original size of the probes, whereas bands a–e correspond to the fragments protected by the liver acetyl-CoA carboxylase mRNA (lanes 2 and 4). None of the species a–e are protected when liver RNA from the fasted rat is used in the S1 nuclease analysis (not shown). In the experimental results shown in Fig. 5, the fragments protected by mammary gland RNA are included for comparison (lanes 1 and 3) because they can be used to interpret the results obtained with the liver RNA. How these specific primers are protected by different forms of acetyl-CoA carboxylase mRNA is schematically summarized in Fig. 4. For example, the FL63 type of acetyl-CoA carboxylase mRNA would completely protect the cDNA segment present in the FL63 probe (band c, 227 nucleotides) and only the first 173 bases of the FL63 probe (band b, lanes 3 and 4). On the other hand, acetyl-CoA carboxylase mRNA of the FL63 type would protect only the first 173 bases of the FL63 probe (band b in lanes 1 and 2) and the full length cDNA included in the FL63 probe (band e, 303 nucleotides). In other words, the protection of 227 nucleotides of FL63 probe (band c) and 173 nucleotides of the FL63 probe (band b) is due to the presence of the FL63 type of acetyl-CoA carboxylase mRNA. The protection of band e with FL63 probe and band b with FL63 probe is due to the presence of the FL63 type of acetyl-CoA carboxylase mRNA. These experiments allow us to conclude that liver RNA contains the FL63 type of acetyl-CoA carboxylase mRNA and only trace amounts, if any, of the FL63 type of acetyl-CoA carboxylase mRNA.

Since the specific activities of these probes and the amounts of RNA used in these experiments were the same, the autoradiographic intensities of the protected segments reflect the relative amount of the specific mRNA in the RNA preparation. Thus, in the case of the FL63 type acetyl-CoA carboxylase mRNA, the most abundant species in the mammary gland, a strong band b with the FL63 probe (Fig. 5, lane 3) should be accompanied by an equally strong band c with the FL63 probe and a much dimmer band b with the same probe. The FL63 type of acetyl-CoA carboxylase mRNA is obviously present in the liver, since the same set of bands is protected by liver RNA. However, the relative intensities of the bands composing this set (fainter band c and stronger band b with the FL63 probe) indicate that this form of acetyl-CoA carboxylase mRNA is not the predominant species in liver. The fact that band b stands out as the best protected fragment with both the FL63 and the FL63 probes indicates that the major acetyl-CoA carboxylase mRNA species in liver has portions of its nucleotide sequence that are identical to the FL63 and FL63 types of acetyl-CoA carboxylase mRNA. These common sequences extend to the insertion point of the 61-base insert peculiar to FL63; they do not include either the 61-base insert (Fig. 1, segment II) or the (C + G)-rich region (Fig. 1, segment IA) of the FL63 type of acetyl-CoA carboxylase mRNA. Therefore, the major species of liver acetyl-CoA carboxylase mRNA contain their own specific sequences at their 5’ ends.

Cloning of the Liver Primer Extension Product A: pAU Clones—In order to characterize the structure of the major forms of acetyl-CoA carboxylase mRNA in the liver, we have cloned primer extension product A (Fig. 2), which is generated by the largest and most abundant species of acetyl-CoA carboxylase mRNA in the liver. A cloning strategy was devised that would selectively enrich a size-selected primer extension product, as described under “Experimental Procedures.” A total of 23 positive clones (pAU clones) was detected among the 440 recombinant clones screened. Restriction endonuclease analysis indicated that all of these clones had the same ~440-base pair insert.
The DNA sequences of the inserts that are present in the pAU clones (Fig. 6) showed all the features that were expected from the primer extension product and our cloning strategy. Specifically, their 3' ends finished at the first residue of primer 263 and they contained poly(T) tails at their 5' ends. These independent clones, differing in the lengths of the poly(T) tails, have identical cDNA nucleotide sequences. The sequence alignment of pAU clones with λFL56 and λFL63 confirms that their regions of sequence identity are exactly confined to the 173 nucleotides located between the start of primer 263 and the insertion point of λFL63's 61-base insert. The 242 nucleotides upstream to this segment constitute the novel 5' end leader of band A acetyl-CoA carboxylase mRNA.

Conclusive evidence that the clone pAU represents the full length cDNA of the liver extension product A was obtained through specific primer extension and cDNA S1 nuclease protection experiments. Three primers covering different extents of the pAU clone were synthesized: primers 263/H2/Taq, 263/FL56/RV, and 263/pAU/RI are 108, 155, and 275 nucleotides long, respectively (Fig. 1). Their 5' ends are aligned to the same point in acetyl-CoA carboxylase mRNA but their 3' ends span into distinct regions upstream of the acetyl-CoA carboxylase mRNA (Fig. 6). The result of the extension of these primers on rat liver RNA is shown in Fig. 7A. Primer 263/H2/Taq (lane 1) gave the complete set of primer extension products: bands A-D and a and b, while the extension of the more template selective primer 263/FL56/RV (lane 2) did not include a product corresponding to band B. The mRNA selectivity of primer 263/FL56/RV indicates that the template producing band B differs from the template producing bands A, C, and D by the exclusion of the bases belonging to segment III (Fig. 1). The extension of the pAU-specific primer 263/pAU/RI produced bands A, C, and D, confirming that the pAU clones were derived from primer extension product A and that primer extension products C and D are shorter versions of A.

Further information about the structures of bands A-D was obtained from cDNA S1 nuclease analysis. The pAU cDNA-specific probe 263/AU/Bam was synthesized for these experiments (Fig. 4); this probe is 458 bases long and includes seven nucleotides from the cloning vector and 38 bases from the cloning poly(T) tail of pAU. These 43 extra bases are important in the recognition of bona fide S1 nuclease-protected fragments. The position of migration of this probe is marked as band e in Fig. 7B, while the fragments that are protected from S1 nuclease by liver RNA from refed animals are identified as bands a (~128 nucleotides), b (173 nucleotides), c (355 nucleotides), and d (415 nucleotides). For comparison, the extension products generated by using primer 263/H2/Taq are also shown in Fig. 7B (lane 1). The correspondence of band sizes and intensities between the primer extension and the S1 nuclease experiments allows us to conclude that the acetyl-CoA carboxylase mRNA template producing primer extension product A and the S1 nuclease-resistant band d is fully cloned in pAU clones. Therefore, the acetyl-CoA carboxylase mRNA form producing the S1 nuclease-resistant band c is a shorter version of the full length pAU type and is very likely the one responsible for primer extension product C. The structure of the acetyl-CoA carboxylase mRNA species producing the many minor primer extension products in the range of 309 nucleotides (including band D) cannot be conclusively identified by these experiments. However, it is very likely that they are the result of incomplete extension of the full length pAU type acetyl-CoA carboxylase mRNA and not the result of full length extension of independent shorter forms, because the S1 nuclease experiment did not reveal any protected fragments matching their sizes.

On the other hand, we can speculate about the structure of the acetyl-CoA carboxylase mRNA producing band B as being an acetyl-CoA carboxylase mRNA species in which the first 242 bases of the pAU type of acetyl-CoA carboxylase mRNA are spliced together with exon H2. This hypothesis is based on the following observations: 1) band B is approximately 47 nucleotides shorter than band A, the same length as the fragment linking exon H2 and the 242-base long pAU-specific leader segment; 2) the inclusion of sequences belonging to this linking fragment in primer 263/FL56/RV results in the disappearance of band B from the set of this primer extension product; 3) the presence of an S1 nuclease-resistant fragment (band a in Fig. 7B) of ~128 nucleotides in length; and 4) the overlapping faint RNA sequencing ladder in the background...
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DISCUSSION

The present studies revealed that the diversity of structural motifs exhibited by the 5' end untranslated region of rat acetyl-CoA carboxylase mRNA is more profuse in liver than in mammary gland. Among the several approaches available to disclose the structure of these liver forms of acetyl-CoA carboxylase mRNA, cloning of the primer extension products is most likely to lead to unambiguous results. Direct sequencing of any mixture of mRNAs poses not only technical difficulties but also results in useless sequence data composed of multiple overlapping ladders. Even in the event that one obtains a partial sequence from which oligonucleotide probe could be designed, the need to generate an appropriate cDNA library from which the rest of the mRNA sequence could be established still remains. Therefore, we directly cloned the primer extension product detected as band A in Fig. 2, following the procedure described under "Experimental Procedures." A number of positive clones (pAU clones) resulted from this approach. DNA sequencing of two of these clones, pAU3 and pAU4, showed that after offsetting the size of the cloning insert, i.e., the expected size for a full length copy of primer extension product A. Supporting experimental evidence that the cDNA in pAU4 is indeed derived from primer extension product A was provided by the specific primer extension and cloning experiments using RNA prepared from different rat tissues under various dietary conditions.

Fig. 8 shows the migration patterns of primer extension products and S1 nuclease-protected fragments from the linked primer extension/S1 nuclease digestion (lanes s) using primer 263/H2/Tag. Mammary gland RNA (lanes 2) gave the FL56-derived primer extension products a and b (open arrows), while liver RNA from animals that have been fasted and refed a fat-free diet generated, as expected, products A-D from the pAU types of acetyl-CoA carboxylase mRNA (black arrows, denote band A) in addition to those from the FL56 type (lanes 3). RNA prepared from livers of rats fed a normal diet produced only bands a and b, which are derived from the FL56 type, in barely detectable amounts (lanes 5). On the other hand, the RNA prepared from adipose tissue of the same rats resulted in large amounts of pAU-derived bands A-C and only traces amounts of FL56's bands a and b (lanes 6). The tRNA, which served as a negative control (lanes 1), and starved liver RNA (lanes 4), which has negligible amounts of acetyl-CoA carboxylase mRNA, produced no acetyl-CoA carboxylase-specific primer extension products. These results indicate that the physiological state plays an important role in controlling the tissue-specific levels of different acetyl-CoA carboxylase mRNAs.
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The 61-base insert of FL63, and pAU provides evidence of the unmistakable modular nature of the 5'-untranslated region. This modular nature was initially manifested by the cDNA S1 nuclease protection patterns and the selectivity of the primer extension products generated by cDNA specific primers. These landmarks of the 5' end of acetyl-CoA carboxylase mRNA permit us to separate it into discrete segments, as shown in Fig. 1. Segment IA designates the (C + G)-rich, 5' end portion of FL65 and FL63, the putative first exon of the mammary gland's acetyl-CoA carboxylase transcriptional unit. Segment II corresponds to the 61-base long putative shuffling exon present in the FL63 type of acetyl-CoA carboxylase mRNA. Segment III is the 47-base string located upstream of exon H2; it is present in all of the cloned forms of acetyl-CoA carboxylase mRNA. However, as explained under “Results,” specific primer 263/FL56/RV extension experiments (Fig. 7A), together with the appearance of the 128-base long fragment in the cDNA S1 nuclease experiments (Fig. 7B, band a) and the background RNA sequence in Fig. 3B, clearly establish the existence of acetyl-CoA carboxylase mRNA forms having the 242-base long pAU 5' end leader (segment IB), but lacking segment III. Thus, the putative nature of segment III as an independent exon in the acetyl-CoA carboxylase gene is strongly emphasized. Segment IV corresponds to the exon in genomic clone H2; it contains the initiation codon of the acetyl-CoA carboxylase coding region. Segment IB designates the first 242 nucleotides of the liver acetyl-CoA carboxylase mRNA form cloned in pAU; it replaces segments IA and II in the acetyl-CoA carboxylase mRNA types discovered in the liver. Segment IB does not affect the acetyl-CoA carboxylase coding region because it does not extend the coding region open reading frame. It is very likely that segment IB originates from the first exon(s) of this novel acetyl-CoA carboxylase transcriptional unit found in the liver.

There are numerous examples of alternative use of exons and promoters as a mechanism to introduce new modes of control of gene expression and to generate diversity from single genes (24-25). The modular nature of the acetyl-CoA carboxylase mRNA 5' end is very suggestive of differential splicing of the acetyl-CoA carboxylase transcriptional unit. This suggestion is further reinforced and complicated by the discovery of new forms of acetyl-CoA carboxylase mRNA with totally distinct 5' ends. The fact that the acetyl-CoA carboxylase mRNA forms present in liver exhibit two mutually exclusive 5' end sequences (segment IA ± II or segment IB in Fig. 1), while sharing the same set of 3' end segments (Fig. 1, segments III and/or IV), implies that in addition to the necessary alternative usage of exons, there may be an alternative usage of promoters in the transcription of the acetyl-CoA carboxylase gene in the rat liver. Since there is only one copy of the acetyl-CoA carboxylase gene per haploid chromosomal set (5), one would have to postulate a hitherto unknown "splicing" mechanism for the generation of both pAU and FL types of acetyl-CoA carboxylase mRNA if there were only one promoter for the acetyl-CoA carboxylase gene. Recently, we have obtained supporting evidence for multiple promoters in the acetyl-CoA carboxylase gene. 3

\[^3\] Luo, X., Park, K., Lopez-Casillas, F., and Kim, K.-H. (1989) Proc. Natl. Acad. Sci. U. S. A., in press.
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Structs were transfected into 30A-5 preadipocytes, both of these DNAs were able to stimulate the expression of chloramphenicol acetyltransferase activity. Further experiments are being carried out to establish that these two DNA fragments are indeed the promoters that are responsible for the generation of the heterogeneous acetyl-CoA carboxylase mRNAs. Interestingly, the results in Fig. 8 suggest that such an alternative usage of promoters may be tissue-specific and sensitive to the dietary manipulations that regulate lipogenesis. The pAU forms of acetyl-CoA carboxylase mRNA are virtually absent in the mammary gland, even at the peak of lactation (lanes 3), as well as in the liver of rats fed a standard diet. Under these conditions the predominant form of acetyl-CoA carboxylase mRNA corresponds to the FL56 type, presumably a "housekeeping" kind of transcript. On the other hand, the pAU forms of acetyl-CoA carboxylase mRNA are the predominant species in the liver upon dietary treatment that maximizes lipogenesis (2) and acetyl-CoA carboxylase mRNA levels (3), suggesting that such a transcriptional unit is the one responsive to changes in the demand for fatty acid biosynthesis. Interestingly, adipose tissue from rats fed a complete diet have minimal amounts of the FL56 type of acetyl-CoA carboxylase mRNA and substantial amounts of the pAU type of acetyl-CoA carboxylase mRNA, implying that tissue-specific controls exist in addition to the mechanisms regulating the hepatic expression of two acetyl-CoA carboxylase gene promoters.

Independently of the actual mechanism giving rise to the 5′ end heterogeneity of acetyl-CoA carboxylase mRNA, the fact that the heterogeneity is confined to the untranslated region of the mRNA opens the possibility that these diverse forms of acetyl-CoA carboxylase mRNA are subjected to some form of translational control (22, 26–29). Work is in progress in our laboratory that will address the many questions posed by the present studies.

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