 Regulation of Rat Pyruvate Carboxylase Gene Expression by Alternate Promoters during Development, in Genetically Obese Rats and in Insulin-secreting Cells

MUltIPLE TRANSCRIPTS WITH 5'-END HETEROGENEITY MODULATE TRANSLATION*

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A previous study on the gene structure of rat pyruvate carboxylase revealed that two tissue-specific promoters are responsible for the production of multiple transcripts with 5'-end heterogeneity (Jitrapakdee, S., Booker, G. W., Cassady, A. L., and Wallace, J. C. (1997) J. Biol. Chem. 272, 20522–20530). Here we report transcription and translation regulation of pyruvate carboxylase (PC) expression during development and in genetically obese rats. The abundance of PC mRNAs was low in fetal liver but increased by 2–4-fold within 7 days after birth, concomitant with an 8-fold increase in the amount of immunoreactive PC and its activity and then decreased during the weaning period. Reverse transcriptase-polymerase chain reaction analysis indicated that the proximal promoter was activated during the suckling period and reduced in activity at weaning. In genetically obese Zucker rats, adipose PC was 4–5-fold increased, concomitant with a 5–6-fold increase in mRNA level. Reverse transcriptase-polymerase chain reaction analysis also showed that the proximal promoter was activated in the hyperlipogenic condition. Conversely, transcription of the proximal promoter was not detectable in various liver cell lines, suggesting that this promoter was not functional under cell culture conditions. In rat pancreatic islets and insulinoma cells, only transcripts D and E, generated from the distal promoter of the PC gene, were expressed. Glucose increased PC transcripts from the distal promoter when the insulinoma cells were maintained in 10 mM glucose. We conclude that the proximal promoter of the rat PC gene plays a major role in gluconeogenesis and lipogenesis, whereas the distal promoter is necessary for anaplerosis. In vitro translation and in vivo polysome profile analysis indicated that transcripts C and E were translated with similar translational efficiencies that are substantially greater than that of transcript D, suggesting that 5'-untranslated regions play a role in translational control.

Pyruvate carboxylase (PC1; EC 6.4.1.1) catalyzes the ATP-dependent carboxylation of pyruvate to oxaloacetate, the first regulated step in the gluconeogenic pathway from pyruvate and its precursors. Mammalian PC is a mitochondrial enzyme that plays a vital role in gluconeogenesis in liver and kidney. PC is also involved in lipogenesis, enabling acetyl group export from mitochondria as citrate for the cytoplasmic biosynthesis of fatty acids in adipose tissue and lactating mammary gland (1). In astrocytes, PC plays an anaplerotic role in replenishing citric acid cycle intermediates used in the biosynthesis of glutamine, which is subsequently converted to glutamate, aspartate, and γ-aminobutyric acid in neurons (2). Recent studies have shown that PC contributes to glucose-induced insulin secretion from pancreatic islet cells (3) by participating in a pyruvate-malate shuttle across the mitochondrial membrane to produce cytosolic NADPH (4).

Short term regulation of PC activity can be achieved by an allosteric regulator, acetyl-CoA, whereas long term regulation involves changes in the total amount of PC through alterations in the rate of enzyme synthesis in liver, kidney, and adipose tissue (5). It has long been demonstrated that PC activity is affected under different physiological and pathological circumstances, e.g. neonatal development, diabetes, nutritional alterations (5), and genetic obesity (6). Although cDNA sequences encoding PC from mammals have been cloned (7–12), little is known about the molecular basis of PC expression in vivo. Cell culture studies have shown that the differentiation of mouse 3T3-L1 fibroblasts to mature adipocytes is accompanied by an increase in the level of PC mRNA and the rate of enzyme synthesis (13–15).

Our studies on the isolation of cDNA (11) and the gene structure (16) of rat PC indicated that although there is only one copy of the PC gene (17), it generates several forms of PC mRNA, which diverge at their 5'-untranslated regions (UTR) but share the same open reading frame encoding a 1178-residue polypeptide (12). Two tissue-specific promoters are responsible for the production of two primary transcripts, which then are differentially spliced to five mature transcripts (16). Transcripts generated from the proximal promoter are restricted to gluconeogenic and lipogenic tissues, whereas transcripts generated from the distal promoter are expressed in a wide variety of tissues (12). Key questions that remain unanswered are...
which promoter of the rat PC gene is activated or repressed under different physiological conditions and whether the 5'-UTRs of different PC mRNAs can modulate their expression.

To gain a further insight into the molecular mechanisms that regulate PC expression in vivo, we have assessed the role of transcriptional regulation during postnatal development and in genetically obese rats by means of a reverse-transcriptase polymerase chain reaction (RT-PCR) analysis that identifies each one of the multiple forms composing the PC mRNA population. We have also shown the 5'-UTRs of different PC mRNAs modulate translation of PC both in vitro and in vivo.

**EXPERIMENTAL PROCEDURES**

**Animals**—Sprague-Dawley rats were bred and housed at a constant temperature of 25 °C in the animal house, University of Adelaide, and were subjected to treatments approved by the Animal Ethics Committee of the University of Adelaide. Litters of 8- or 12-week-old Zucker rats were obtained from Monash University, Clayton, Victoria. Tissues were quickly removed from the sacrificed animals, snap-frozen in liquid nitrogen, and stored at -80 °C.

**Cell Culture**—The rat cell lines used were: BRL3A (ATCC: CRL 1442), Reuber H35 (ATCC: CRL 1548), MA-RH77 (ATCC: CRL 1601), L6 myoblast (ATCC: CRL 1458), rat mammary gland carcinoma cell line (obtained from Institute of Medical and Veterinary Science, Adelaide, Australia), FT03 (obtained from the Institute of Cell and Tumor Biology, Heidelberg, Germany), fetal rat liver hepatoma cell line (FRL4.1; gift from Dr. G. Yeh, Department of Biochemistry, University of Western Australia, Nedlands), all of which were routinely grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 mg/ml streptomycin, and 100 units/ml penicillin at 37 °C. Bovine insulinoma (INS-1) was obtained from the endocrine unit of the University of Adelaide. Litters of 8- or 12-week-old Zucker genetically obese rats by means of a reverse-transcriptase polymerase chain reaction (RT-PCR) analysis that identifies each one of the multiple forms composing the PC mRNA population. We have also shown the 5'-UTRs of different PC mRNAs modulate translation of PC both in vitro and in vivo.

**Ribonuclease Protection Assay**—The pRPC3 plasmid was constructed by subcloning a 0.3-kilobase pairs SacI-PstI fragment of the RACE (Anchor/PC 2) PCR product amplified from rat PC cDNA (11) to SacI-PstI-digested pBluescript SK (Stratagene). The β-actin cDNA (13) was constructed by RT-PCR as follows. Rat brain RNA was annealed to a cDNA synthesis primer designed from the coding region of rat β-actin mRNA (5'-TCTTGATGTGTTGCCAAGGCCAG-3') (18) and reverse-transcribed. The cDNA was subjected to PCR with a sense primer (β-actin (+)) (5'-AACTTGGATCCCTGGAGAGAGCTATGAC-3' with BamHI restriction site attached at the 5'-end, underlined) and an antisense primer (β-actin (-)) (5'-TCCCGGTACCCAGACGACTTGTTGGCGA-3'; with a KpnI restriction site attached at the 5'-end, underlined) (18). The PCR products were double-digested with KpnI and BamHI, cloned into KpnI-BamHI-digested pBluescript, and sequenced.

The above plasmids were linearized by digestion with EcoRI and BamHI, respectively, and full-length probes were gel-purified. The antisense riboprobe was synthesized using the MaxiScript kit (Ambion, TX) with T7 RNA polymerase in the presence of 50 μCi of [α-32P]UTP (8000 mCi/mmol). The full-length probe was gel-purified and eluted. Total RNA was isolated from frozen tissue using an RNAqueous kit (Ambion, TX), and ribonuclease protection analysis was performed using a ribonuclease protection assay kit (Ambion, TX). Briefly, 10 μg of total RNA were hybridized with approximately 1 × 107 cpms of each probe at 45 °C for 18 h. The probe and unhybridized RNAs were digested with RNase A and T1 for 30 min at 37 °C. The 201-bp protected fragment representing β-actin mRNA and the 341-bp protected fragment representing the common coding region of different PC mRNAs (see Fig. 1) were denatured and separated by electrophoresis on 6% acrylamide, 8 μa urea gel at 350 V for 2 h. The gel was dried and placed in the PhosphorImager screen overnight. The intensities of hybridization bands were quantitated by PhosphorImager analysis using the ImageQuant Software (Molecular Dynamics) and corrected for uneven loading of the RNA samples by comparison with β-actin bands.

**Reverse Transcriptase Polymerase Chain Reaction**—The first strand cDNA for PCR was synthesized as follows. 10 μg of total RNA were hybridized with 100 ng each of PC-8 primer (5'-GGACCTGGAAGCGTGT-3') (positions +484 to +503) (11) and of β-actin 1 primer (as described above) and reverse-transcribed as described previously (12). The PCR primers were as follows: 5'-GGGGAATTCGGCGAACACCTC-3' (MRACE II), which was directed to a common coding sequence of different PC mRNAs (see Fig. 1) were denatured and separated by electrophoresis on 6% acrylamide, 8 μa urea gel at 350 V for 2 h. The gel was dried and placed in the PhosphorImager screen overnight. The intensities of hybridization bands were quantitated by PhosphorImager analysis using the ImageQuant Software (Molecular Dynamics) and corrected for uneven loading of the RNA samples by comparison with β-actin bands.

**Preparation of Tissue Homogenate and PC Assay**—One g of frozen liver was ground to a powder in liquid nitrogen and homogenized in 4 volumes of extraction buffer (0.25 M sucrose, 0.1 mM EDTA, 20 mM Tris-HCl, pH 7.2, 0.5 mM diethiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride) at 4 °C. The homogenate was freeze-dried and reconstituted in 3 ml of 50 mM Tris acetate, pH 7.0, 5 mM ATP, 5 mM MgCl2, and 0.5 mM EDTA. The homogenate was centrifuged at 20,000 × g for 20
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min at 4 °C, and the supernatant-containing PC was kept at ~80 °C. For adipose tissue, 1–2 g of epididymal fat pads were homogenized as described above, and fat was removed from the homogenate by centrifugation at 10,000 × g for 5 min at 4 °C. The aqueous extract was removed from the cell debris, freeze-dried, and subsequently reconstituted as described above. Twenty micrograms of protein (19) were assayed for PC activity at 30 °C for 2 min by a radioactive CO2 fixation (20), except that the reaction was coupled with the conversion of oxaloacetate to malate by 2 units of malic dehydrogenase (Sigma) in the presence of 0.1 mM NADH. One unit of PC is defined as the conversion of 1 μmol of NaH14CO3 to malate per min, and the PC activity is defined as milli-
units/mg of total protein.

SDS-Polyacrylamide Gel Electrophoresis and Western Analysis—Total protein from liver homogenate (20 μg) was subjected to reducing discontinuous SDS-polyacrylamide gel electrophoresis on a 4% stacking gel and 7.5% polyacrylamide-separating gel (21). The separated proteins were transferred to nitrocellulose filters using a semi-dry electrotblotter (Multiphor II Novablot, Amersham Pharmacia Biotech). Membranes were blocked with 1% bovine serum albumin in 10 mM tris-blotter (Multiphor II Novablot, Amersham Pharmacia Biotech). Proteins were transferred to nitrocellulose filters using a semi-dry electrophoresis at 10,000 g for 5 min. One ml of cytosolic supernatant was then titrated with RNAs in RT-PCR and found that using a 1:100 dilution of cDNA synthesized from 10 μg of starting RNA gave reliable results (data not shown). In 20-day fetal liver, transcript D, the major form transcribed from the distal promoter was detectable in a greater abundance than transcript C, transcribed from the proximal promoter (Fig. 3). The level of transcript C was increased in 1-day and 7-day pups, respectively. It is interesting to note that although transcript D was accumulated in adults concomitant with a decrease of transcript C (Fig. 3), the level of PC and its activity were decreased (Fig. 2, A and C). This suggested that the translation of transcript D produced during such a period is less efficient than transcript C. However, transcripts A and B, minor forms transcribed from the proximal promoter, were not detectable, and this is consistent with our previous study (12).

Overexpression of PC in Genetically Obese Rats—In genetically obese Zucker rats, it has been reported that PC levels are 2–5-fold increased in the adipose tissue of obese animals (ob/ob) (6). To investigate whether this increase in the amount of PC is because of an enhanced transcriptional activity of the gene, we performed ribonuclease protection assays and RT-PCR experiments to examine the transcriptional activity of PC in obese rat adipose tissue. The obese rat was chosen for this study because it is known to have an obesogenesis phenotype (characterized by increased body weight and fat accumulation) and a high PC activity (7). We found that the levels of PC transcripts (transcripts A, B, and C) were increased 2-fold and 4-fold in 1-day and 7-day pups, respectively (Fig. 2C). However, the increases in the amounts of PC transcripts are not as dramatic as those observed in PC protein (4-fold and 8-fold), suggesting that translational control might play a role in accelerating the rate of enzyme synthesis. The abundance of PC transcripts is also decreased during weaning and in adults, as seen with the level of PC protein and PC activity (Fig. 2, B and C).

Because the rat PC mRNAs are composed of multiple transcripts generated from alternate promoters (16), the RNase protection assay results only reflect an overall transcriptional activity of the gene. Since the sizes of the 5'-UTRs of different rat PC mRNA isoforms are relatively small compared with the common coding region of the messages (4.0 kilobase pairs), conventional Northern analysis using a probe synthesized from the coding region cannot differentiate between these subtle size differences. Therefore, semi-quantitative RT-PCR was also performed to identify which promoter of the PC gene was being used to control the expression. Two sense primers directed against the most 5'-untranslated region of class I (exon 1B) and class II (exon 1D) PC transcripts (12, 16) and an antisense primer directed to a common coding region of the different transcripts were used to selectively amplify by PCR the 5'-ends of the different PC cDNA species (see Fig. 1). We initially titrated amounts of RNAs in RT-PCR and found that using a 1:100 dilution of cDNA synthesized from 10 μg of starting RNA gave reliable results (data not shown). In 20-day fetal liver, transcript D, the major form transcribed from the distal promoter was detectable in a greater abundance than transcript C, transcribed from the proximal promoter (Fig. 3). The level of transcript C was increased in 1-day and 7-day pups, respectively. It is interesting to note that although transcript D was accumulated in adults concomitant with a decrease of transcript C (Fig. 3), the level of PC and its activity were decreased (Fig. 2, A and C). This suggested that the translation of transcript D produced during such a period is less efficient than transcript C. However, transcripts A and B, minor forms transcribed from the proximal promoter, were not detectable, and this is consistent with our previous study (12).

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PCR as described above. In adipose tissues of obese rats, PC transcripts were increased 5- to 6-fold higher than those of their lean litter mates, whereas \(-\)actin message was not affected (Fig. 4A). RT-PCR clearly showed that the increase in mRNA was mainly because of an increase in transcript C with a decrease in transcripts D and E between the two groups of rats (Fig. 4B). In livers from the obese rats, PC transcripts were also increased. A 4-fold difference was observed between the two groups of animals (see Fig. 4A). RT-PCR analysis revealed the increase in hepatic PC mRNA again resulted from an increase in transcript C (see Fig. 4B) with little change in transcripts D and E.

The increase in PC transcripts in both adipose tissue and liver was concomitant with an increase in the amount of PC detected by Western immunoblot. As shown in Fig. 4C, the levels of adipose PC in obese rats were \(2-3\)-fold higher than in their lean litter mates.

**Expression Pattern of PC Transcripts in Cultured Cells and in Pancreatic Islets**—Although PC was detected by Western immunoblot in every cell line (data not shown), the expression patterns of PC transcripts in liver cell lines were different from that of an intact liver. Transcript C generated from the proximal promoter was not detectable even though 100 times more cDNAs were used in RT-PCR, but transcripts D and E transcribed from the distal promoter (12) were detectable by RT-PCR (Fig. 5A). It is unlikely that the primer binding site lo-
transcripts C, D, and E, respectively, were analyzed by RT-PCR with PC primers and \( \beta \)-actin primers (internal control). D and E, transcripts D and E, respectively.

**DISCUSSION**

Gluconeogenesis does not begin in the livers of rats until immediately after birth (25). Of the four gluconeogenic enzymes present in liver (PC, phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase), PC and phosphoenolpyruvate carboxykinase are present in the liver at negligible levels before birth but appear rapidly after birth (20, 23), consistent with the onset of gluconeogenesis. Although the mechanisms involved in phosphoenolpyruvate carboxykinase induction have been studied intensively (26), the mechanisms that control the production of PC during postnatal development are unknown but are potentially com-

**FIG. 4.** Expression of PC in lean and obese Zucker rats. Livers and adipose tissues from 12-week lean (\( n = 4 \)) and obese (\( n = 4 \)) rats were analyzed by ribonuclease protection assay (A), RT-PCR (B), and Western immunoblot (C). The results for only two animals of each group are shown in the figure. C, D, and E, transcripts C, D, and E, respectively.

**FIG. 5.** RT-PCR analysis of PC mRNA isoforms in different rat cell lines. Total RNAs from liver cells (BRL3A, FRL4.1, FT03, McA-RH777, and Reuber H35), myoblast (L6), and mammary gland carcinoma (INS-1) cells (24). RT-PCR was performed on RNA extracted from these two cell types. Only transcripts D and E were detected in both cell types (see Fig. 5B), suggesting that the distal promoter plays a role in anaplerosis. To test whether glucose can increase transcription from the distal promoter, INS-1 cells were initially maintained in RPMI medium containing 1 mM glucose for 48 days and then maintained in the same glucose-free media supplemented with 1, 10, and 20 mM glucose. Glucose (10 or 20 mM) dramatically increased the level of transcripts D and E in INS-1 cells (Fig. 5B).

**5'-UTRs of Different PC Transcripts Modulate Their Translation**—A possible role of the different 5'-UTRs could be to determine the translational efficiency of the mRNA species. We carried out experiments using both *in vitro* translation with reticulocyte lysate system and *in vivo* polysome profile analysis.

Because of the difficulty in constructing a full-length rat PC cDNA clone from different lambda clones and PCR fragments due to a lack of convenient restriction sites to join them together, we generated three constructs that contained different 5'-UTR (95 bp, transcript C; 96 bp, transcript D; and 59 bp, transcript E) (12), followed by an open reading frame encoding the 490 residues of the biotin carboxylation domain plus the 3'-UTR and poly(A) tail of rat PC mRNA (Fig. 6A). When translated with reticulocyte lysate, each mRNA transcribed from the above constructs yielded a single protein band of molecular mass approximately 51 kDa, as predicted (Fig. 6B). The amounts of the 51-kDa band being translated from transcripts C and E were comparable. However, transcript D yielded the same protein band but with 5–6-fold less efficiency than transcripts C and E.

A polysome profile analysis of different PC mRNA isoforms of 7-day pup livers is shown in Fig. 7. Transcript C was distributed from the bottom to the middle of the gradient, associated with polysome fractions (lanes 1 to 7), suggesting that this transcript is translationally active. Likewise, transcript E was mostly found in the bottom to the middle of the gradient (lanes 3 to 7) but in lower abundance than transcript C. In contrast, transcript D is found primarily in the upper portions of the gradient, indicating a lower degree of translation for this transcript. It is therefore likely that most PC protein will be synthesized from transcript C, which is the predominant form being produced during the immediate postnatal gluconeogenic period. Release of ribosomes from the mRNAs by EDTA treatment caused all the transcripts to be found near the top of gradient (lanes 11 and 12).

**FIG. 6A.** Western immunoblot analysis of different rat liver PC mRNA isoforms with polysome fractions. (A) Livers from 7-day-old rats were homogenized and the polysome profile analysis was performed with the above constructs. (B) The gradient was run in the presence of EDTA to release polysomes. It is evident that most of the mRNAs are found in the upper regions of the gradient, indicating a lower degree of translation for these mRNAs. (C) The polysome profile of the liver PC mRNA isoforms is shown in Fig. 7. Transcript C is translationally active, while transcripts D and E are found mostly in the bottom to the middle of the gradient (lanes 3 to 7). In contrast, transcript D is found primarily in the upper portions of the gradient, indicating a lower degree of translation for this transcript. It is therefore likely that most PC protein will be synthesized from transcript C, which is the predominant form being produced during the immediate postnatal gluconeogenic period. Release of ribosomes from the mRNAs by EDTA treatment caused all the transcripts to be found near the top of gradient (lanes 11 and 12).
complicated, involving switching of two promoters. Transcript C, generated from the proximal promoter, is the major form that is increased during the suckling period, concomitant with an increased amount of PC and its activity. During this period gluconeogenesis occurs at the highest rate (25). This suggests that the proximal promoter of the PC gene is activated to supply the demand of cells producing PC to fully participate in gluconeogenesis during such a period. However, during the weaning period and in adults, when the gluconeogenic rate has decreased, the proximal promoter activity was decreased, and transcript C declined.

In genetic obesity, PC expression is increased as are other lipogenic enzymes (6). Although adipose tissue is the main lipogenic tissue in rats, liver is also active in lipogenesis (27). We have demonstrated that both PC transcripts and protein were also increased in the livers of obese rats at 12 weeks but not at 8 weeks. However, the effects were not as marked in adipose tissue (data not shown), as had also been noted previously for hepatic PC protein (6). Thus it is likely that lipogenesis in the livers of the obese rats was not as active in week 8 as in week 12. Transcript C is the major transcript that is increased in both liver and adipose tissue of the obese rats, suggesting that the proximal promoter is transcriptionally activated during the lipogenic period. The presence of a putative fat-specific element 1 on the proximal promoter (16) suggests that overexpression of PC might also be mediated through this motif, as occurs in the fatty acid synthetase gene (28). Insulin selectively inhibits the expression of the proximal promoter of rat PC (16). However, the activity of this promoter is increased in the adipose tissue of the obese Zucker rat (Fig. 5A), even though blood insulin levels are known to be dramatically increased in this model of diabetes (29, 30). Activation of the proximal promoter of PC rather than its inhibition is another manifestation of the insulin resistance of the obese Zucker rat.

An anaplerotic role for PC in rat pancreatic islets (3, 31) and INS-1 cells (24) has recently been proposed because higher physiological concentrations of glucose rapidly stimulate insulin secretion by the islets and also cause an increase in PC expression in the islet (3, 32). The high level of PC permits the rapid formation of oxaloacetate, which serves as a substrate for the pyruvate/malate shuttle across the inner mitochondrial membrane, thus providing cytosolic NADPH (4). In this study we found that only transcripts D and E were detected in pancreatic islets and INS-1 cells, suggesting that the distal promoter of the rat PC gene is responsible for anaplerosis in these cells. Glucose also increased PC transcription from the distal
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promoter when INS-1 cells were maintained in 10 mM glucose. The same concentration of glucose was also reported to enhance PC transcription rate in pancreatic islets (3).

The absence of transcript C produced from the proximal promoter in every liver cell line indicates that under these cell culture conditions the regulation of PC expression is different from in vivo conditions. Because the proximal promoter is turned on during gluconeogenesis in vivo, this finding is consistent with the observation that under cell culture conditions, gluconeogenesis occurs only in primary cultures of hepatocytes and perfused rat livers (33, 34).

It is apparent that the proximal promoter of the rat PC gene is inducible during gluconeogenesis and lipogenesis, whereas the distal promoter is important for anapleosis. During the suckling period, when gluconeogenesis is accelerated, the proximal promoter is activated, resulting in an increase in the amount of transcript C. In vitro translation and in vivo polysome profile analysis clearly showed that translation efficiency of transcript C is higher than transcript D and hence would result in more PC protein being produced during this period. Activation of transcription from the proximal promoter instead of the distal promoter of PC gene might be a key mechanism to allow an independent regulation. We have previously shown that both promoters contained different putative transcription factor binding sites and responded to insulin differently (16). Because different PC transcripts only differ in their 5′-UTRs, different translation efficiencies of these mRNAs isoforms must be mediated through the 5′-UTR. It is noted (35) that the 5′-UTR of many mRNAs have a tendency to form a secondary structure, which could prevent ribosomal access to the 5′-end. Computer-assisted analysis of secondary structure prediction revealed that 5′-UTR of transcript D can potentially form a stable hairpin structure due mainly to pairing between sequences derived from exon 1C and 1D. However, removal of the sequence derived from exon 1C sequence, which is naturally spliced out in transcript E, abolished this structure (data not shown). Whether secondary structure features contribute to the low translation efficiency of transcript D must await in vitro mapping data and phylogenetic studies.

It is interesting to note that acetyl-CoA carboxylase (ACC), which is another member of the biotin carboxylase family and also plays an important role in fatty acid synthesis, shares many regulatory mechanisms in common with PC. For example, multiple ACC mRNA isoforms having the same coding sequence but differing in their 5′-UTRs, have been identified in rat (36, 37). These arise by differential splicing at the 5′-end of transcripts from the single rat ACC gene, which is alternatively transcribed from two distinct promoters (38) that are highly regulated under different physiological conditions (39). Recent studies also proposed that ACC is an important enzyme providing malonyl-CoA as a coupling factor necessary for glucose-induced insulin secretion in pancreatic islets (24, 31). As with PC, only the housekeeping promoter of the ACC gene is functional and activated in pancreatic islets upon glucose-induced insulin secretion (40). Transcripts produced from these two promoters have also been shown to affect protein translation in vitro (41).

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