Generation from a Single Gene of Two mRNAs That Encode the Mitochondrial and Peroxisomal Serine:Pyruvate Aminotransferase of Rat Liver*

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In rat liver there are two types of serine:pyruvate aminotransferase (SPT) whose natures are indistinguishable but whose subcellular localization are different. One is a mitochondrial enzyme (SPTm) and the other a peroxisomal enzyme (SPTp). We compared, in this study, the structure of mRNAs encoding SPTm and SPTp by comparison of the sizes after removal of poly(A) tail by ribonuclease H and by means of RNA blot analysis and S1 nuclease protection assay. No differences were detected between these two mRNAs other than that about 100 nucleotides of the 5'-terminal sequence of SPTm mRNA are lacking in SPTp mRNA, and the length of the poly(A) tail is different. Southern blot analysis of rat genomic DNA showed that the SPT gene is single. Primer extension and S1 nuclease mapping analyses, using a DNA fragment of a genomic clone, revealed that the SPTm and SPTp mRNAs are transcribed from different initiation sites, about 70 nucleotides apart, in the same exon, exon 1. Ribonuclease protection assay performed with RNA hybridization probe corresponding to 5'-terminal portion of SPTm mRNA also showed that the 5'-terminal sequence of SPTp mRNA is about 70 nucleotides shorter than that of hormone-responsive SPTp mRNA. These results indicate that the different organellar distribution of SPTm and SPTp, the products of the same SPT gene, arises from transcription from different initiation sites, conferring N-terminal extension peptide, the mitochondrial targeting signal, only on the translation product of SPTm mRNA.

Serine:pyruvate aminotransferase (EC 2.6.1.51) is localized in two subcellular organelles, mitochondria and peroxisomes, in rat liver (1-3). The mitochondrial enzyme (SPTm) and peroxisomal enzyme (SPTp) have very similar immunological (2,3), catalytic and physical (4) properties, but their responses to hormones or other stimuli are quite different. Only SPTm shows marked induction on the administration of glucagon or insulin to rats (2,5,6), whereas di-(2-ethylhexyl)phthalate (DEHP), a proliferator for peroxisomes (7,8), causes a selective increase in SPTp, as revealed on immunocytochemical analysis (9). SPTm is synthesized on membrane-free ribosomes as a larger precursor (pSPTm), which is specifically translocated into mitochondria in vitro and also in vivo, with the concomitant processing to the mature form of the enzyme (10,11).

In our previous studies (5,6), two types of SPT mRNA with different sizes were detected on RNA blot analysis, with the cDNA fragment for SPTm as a probe. The larger mRNA (approximately 1900 nucleotides) was induced by glucagon and insulin, whereas the smaller one (approximately 1700 nucleotides) was not affected by these hormones, indicating that the 1900-nucleotide mRNA and 1700-nucleotide mRNA code for pSPTm (45 kDa on sodium dodecyl sulfate-polyacylamide gel electrophoresis) and SPTp (43 kDa on sodium dodecyl sulfate-polyacylamide gel electrophoresis), respectively.

In this study, the structure of the SPTm mRNA (1900-nucleotide mRNA) and SPTp mRNA (1700-nucleotide mRNA) were compared in detail, and the transcriptional initiation sites of the two mRNAs were determined to elucidate the origin(s) of the two closely related mRNAs for SPT.

MATERIALS AND METHODS

Preparation of mRNAs—Male rats of the Wistar strain were intraperitoneally administered either glucagon (300 pg/100 g body weight) 3.5 h before sacrifice, insulin (2 units/100 g body weight) 6 h before sacrifice, or DEHP (0.5 g/100 g body weight) daily for 2 or 3 days before sacrifice. Poly(A)* RNA was prepared from the excised livers as described previously (6). In RNA prepared from the livers of glucagon- or insulin-injected rats, SPTm mRNA is more abundant than SPTp mRNA (6). In contrast, SPTp mRNA is more abundant than SPTm mRNA in RNA prepared from the livers of normal rats (5) and rats treated with DEHP.2

Isolation of SPT Genomic Clones—High molecular weight DNA was prepared from rat liver as described by Maniatis et al. (12). A genomic library was constructed according to Hohn and Collins (13) using a cosmid vector, pHCT79, and an EcoRI partial digest. DNA from rat liver was isolated by a standard procedure.

Analytical Procedures—RNA and DNA blot analyses were carried out according to Thomas (14) and Southern (15), respectively, except that nylon membrane was used instead of nitrocellulose membrane.
untranslated region and the coding region for the precursor of SPTm. The 'P-labeled 5'-ends of exon 1 of the SPT gene. The dotted line indicates the sequence of the Sl cleavage assay was performed according to Berk and Sharp (17) using cDNA fragment I and genomic DNA fragment J for both analyses. Hybridization probes (cDNA fragments A-H, Fig. 1) were labeled with [a-32P]dCTP and the Klenow fragment of DNA polymerase I by the random primer labeling method (16). The nu-
clease Sl protection assay was carried out according to the method of Melton et al. (24) using RNA hybridization probes prepared from cDNA clone for pSPTm, pRspt 321, and at the bottom, exon 1 of the SPT gene. The dotted line indicates the sequence of the Okazaki-Berg vector. Narrow and wide boxes indicate the 5'- or 3'-untranslated region and the coding region for the precursor of SPTm (pSPTm), respectively. Hatched boxes show the sequence encoding the N-terminal extension peptide of pSPTm. The 32P-labeled 5'-ends of fragments I and J are denoted by asterisks. The first nucleotide, A, of the ATG triplet encoding the initiation Met of pSPTm is numbered 1. The numbers in parentheses indicate the 3'-terminal nucleotides (nt) generated on enzymic cleavage. For primer extension analysis, an oligonucleotide of 17 bases, which is complementary to nucleotides 134-150 of SPTm cDNA (20), was synthesized using a Model 381A DNA synthesizer, Applied Biosystems. The oligonucleotide was labeled at its 5'-end to a specific reaction of 4-6 x 10^6 cpm/ml and then used as a primer. A mixture (30 ml) of 0.16 pmol of labeled primer and 10 pg of poly(A) RNA in 70 mM NaCl was boiled for 4 min and then cooled to 37 °C for primer-mRNA annealing. The reverse transcriptase reaction (21) and the following procedures (22) were carried out under the reported conditions. The primer-extended product was analyzed by 7 urea, 6% polyacrylamide gel electrophoresis (23).

RNase protection assay was carried out according to the method of Melton et al. (24) using RNA hybridization probes prepared from fragments of a cDNA clone for pSPTm, pRspt 321, and at the bottom, exon 1 of the SPT gene. The dotted line indicates the sequence of the Okazaki-Berg vector. Narrow and wide boxes indicate the 5'- or 3'-untranslated region and the coding region for the precursor of SPTm (pSPTm), respectively. Hatched boxes show the sequence encoding the N-terminal extension peptide of pSPTm. The 32P-labeled 5'-ends of fragments I and J are denoted by asterisks. The first nucleotide, A, of the ATG triplet encoding the initiation Met of pSPTm is numbered 1. The numbers in parentheses indicate the 3'-terminal nucleotides (nt) generated on enzymic cleavage. For primer extension analysis, an oligonucleotide of 17 bases, which is complementary to nucleotides 134-150 of SPTm cDNA (20), was synthesized using a Model 381A DNA synthesizer, Applied Biosystems. The oligonucleotide was labeled at its 5'-end to a specific reaction of 4-6 x 10^6 cpm/ml and then used as a primer. A mixture (30 ml) of 0.16 pmol of labeled primer and 10 pg of poly(A) RNA in 70 mM NaCl was boiled for 4 min and then cooled to 37 °C for primer-mRNA annealing. The reverse transcriptase reaction (21) and the following procedures (22) were carried out under the reported conditions. The primer-extended product was analyzed by 7 urea, 6% polyacrylamide gel electrophoresis (23).

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Materials—Nylon membrane (Hybond-N) and the multiprime DNA labeling system were obtained from Amersham Japan, pd(T)12-15, and polyadenylic acid from Pharmacia Japan, the in vitro packaging extract (Gigapack plus) from Stratagene Cloning System, [γ-32P] ATP (6000 Ci/mmol), [α-32P]dCTP (3000 Ci/mmol), and [α-32P]dATP (800 Ci/mmol) from Du Pont-New England Nuclear, avian myeloblastosis virus reverse transcriptase from Life Science Inc., nuclease S1 from Sankyo Co. (Japan), Sequenase from United States Biochemical, T4 DNA polymerase, SP6 RNA polymerase, and human placenta RNase inhibitor from Takara Shuzo Co. (Japan), and RNase A and RNase T1 from Boehringer Mannheim GmbH (West Germany). Other biochemicals were obtained from the suppliers reported previously (5, 20).

RESULTS

Comparison of SPTm mRNA with SPTp mRNA—In our previous papers (5, 6) it was reported that SPTm mRNA was approximately 200 nucleotides larger than SPTp mRNA, as judged by RNA blot analysis. To compare the net sizes of the SPTm and SPTp mRNAs, the poly(A) tail of these mRNAs was removed by means of oligo(dT)-dependent digestion with RNase H. As shown in Fig. 2, the sizes of SPTm mRNA (about 1900 nucleotides, lanes 2 and 2') and SPTp mRNA (about 1700 nucleotides, lane 1) changed to approximately 1600 nucleotides (lanes 6 and 6') and 1500 nucleotides (lane 5), respectively, on removal of their poly(A) tails. A similar

![Figure 1](http://www.jbc.org/)

**FIG. 1.** DNA fragments used in this study. At the top is the insert of a cDNA clone for pSPTm, pRspt 321, and at the bottom, exon 1 of the SPT gene. The dotted line indicates the sequence of the Okazaki-Berg vector. Narrow and wide boxes indicate the 5'- or 3'-untranslated region and the coding region for the precursor of SPTm (pSPTm), respectively. Hatched boxes show the sequence encoding the N-terminal extension peptide of pSPTm. The 32P-labeled 5'-ends of fragments I and J are denoted by asterisks. The first nucleotide, A, of the ATG triplet encoding the initiation Met of pSPTm is numbered 1. The numbers in parentheses indicate the 3'-terminal nucleotides (nt) generated on enzymic cleavage.

![Figure 2](http://www.jbc.org/)

**FIG. 2.** Removal of the poly(A) tails from the SPTm and SPTp mRNAs. The poly(A) tails were removed from the mRNAs as described previously (6) with some modifications. Poly(A) RNA (5 pg), prepared from the livers of normal rats (odd-numbered lanes) or the livers of glucagon-treated rats (even-numbered lanes), was incubated at 65 °C for 3 min in the presence of 0 μg (--; lanes 3, 4, and 9-14), 1 μg (+; lanes 5-8) or 7 μg (++; lanes 15 and 16) of oligo(dT), and 0 μg (--; lanes 3-10), 2 μg (+; lanes 11 and 12) or 5 μg (++; lanes 13-16) of poly(A). After rapid cooling of the mixtures, KCl was added to a final concentration of 50 mM and then the reaction mixtures were further incubated at room temperature for 15 min. RNase H digestion was performed at 37 °C for 30 min in a 30 μl reaction mixture consisting of 40 mM Tris-HCl (pH 7.6), 4 mM MgCl2, 1 mM dithiothreitol, 4% glycerol, 0.003% bovine serum albumin, 1 unit/μl of RNase A inhibitor (human placenta), and 67 milliunits/μl of RNase H. The digested mRNA was precipitated with ethanol, washed with 80% ethanol, dried, and then subjected to 1.5% agarose gel electrophoresis for RNA blot analysis. Lanes 1 and 2 show the results of usual RNA blot analysis using RNAs with no treatment. Dashed numbers represent shorter exposure (about one-fourth) of respective lanes. 32P-Labeled fragment D was used as a hybridization probe. nt, nucleotides.
reduction in mRNA size was observed even in the absence of exogenous oligo(dT), as indicated in lanes 4, 4', and 3. However, the limited nucleolysis was considered to reflect the oligo(dT)-dependent removal of the poly(A) tail, because the size reduction of mRNAs observed in the absence of oligo(dT) was inhibited by the addition of poly(A) (lanes 11, 12, 12', 13, 14, and 14') and the further addition of oligo(dT) again caused the reduction in mRNA size (lanes 15, 16, and 16'). It appeared that the poly(A)+ RNA preparation used in this experiment contained a significant amount of oligo(dT), probably being contaminated during oligo(dT)-cellulose column chromatography of the total RNA. It was indicated that the size of SPTm mRNA after removal of poly(A) tail was about 100 nucleotides larger than that of SPTp mRNA.

We next examined the similarity of the nucleotide sequences of the SPTm and SPTp mRNAs by means of RNA blot analysis, using fragments A-G, which cover nearly the entire region of SPTm cDNA (see Fig. 1). As shown in Fig. 3, both SPTm mRNA (about 1900 nucleotides) and SPTp mRNA (about 1700 nucleotides) were detected when 32P-labeled fragments B-G were used as hybridization probes. The hybridization band of SPTm mRNA (1700 nucleotides) with fragment B was detectable but rather weakly, because of a lower specific radioactivity of the probe (about one-tenth that of fragments C-G) due to its small size and low content of SPTp mRNA. With fragment A, however, only SPTm mRNA, 1900 nucleotides, was detected. These results suggested that the nucleotide sequences of these two SPT mRNAs are identical or very similar as far as the region corresponding to fragments B-G of SPTm cDNA is concerned but the 5'-terminal sequence corresponding to fragment A is lacking in SPTp mRNA.

To examine in more detail the differences in the structures of SPTm and SPTp mRNAs, an S1 nuclease protection assay was carried out (Fig. 4). When 5'-end-labeled cDNA fragment I (see Fig. 1) was hybridized with an SPTm mRNA-enriched poly(A)+ RNA fraction prepared from the livers of glucagon-treated rats, the band protected against S1 nuclease digestion corresponded to about 1500 nucleotides (Fig. 4, right lane 4). On the other hand, a band corresponding to about 1400 nucleotides was protected when poly(A)+ RNA prepared from the livers of normal (Fig. 4, right lane 3) or DEHP-administered rats (lanes 5 and 6) was used for the assay. No other significant bands corresponding to shorter than 1400 nucleotides were observed. This indicates that there are no mismatches, detectable on S1 nuclease digestion, between the SPTm and SPTp mRNAs, although their 5'-terminal regions are different.

**SPT Gene Is a Single Copy Gene**—To elucidate the mechanism underlying the generation of the two types of SPT mRNA, whose nucleotide sequences are very similar but whose transcriptions are independently regulated, it is necessary to know how many SPT genes there are. Fig. 5 shows the results of Southern blot analysis of genomic DNA extracted from rat liver. Only one band was detected after digestion with all restriction endonucleases used except that no band was detectable after digestion with PvuII (lane 7). Recently, it has been shown, on sequence analysis of an SPT genomic clone, that there are no introns around the region corresponding to fragment C which is located on exon 1. Thus the 103-base pair fragment generated on PvuII digestion of genomic DNA (cf. Fig. 1) may not be retained on the agarose gel. This may be the reason for the detection of no band in lane 7. The data of Southern blot analysis thus strongly suggested that the SPT gene is single, that is, both the SPTm and SPTp mRNAs are transcribed from a single gene. One band with Southern blot analysis alone may not be sufficient as evidence of single gene, but among the 11 restriction nucleases used in the analysis, seven do not have their cleavage site in exons, and all the digested fragments except the PvuII fragment contain introns where more mutation is known to accumulate. If there were two distinct SPT genes, the 5'-upstream region would be expected to be a site of variation, because the responses of SPTm mRNA and SPTp mRNA to hormones are different. Since the region corresponding to fragment C is on exon 1, most of the digested fragments detected in this analysis with fragment C as probe should contain the 5'-upstream region. Therefore, one band with fragment C irrespective of the restriction nucleases used can be taken as good evidence of single gene. In addition, the four SPT genomic clones isolated, cRGsp1-4, were shown described above suggest that SPTm and SPTp mRNAs are generated from a single gene. Recent sequencing analysis of the genomic clone showed that fragments A, B, and C, and a part of D of SPTm cDNA are contained in exon 1 (278 base pairs) in a SPT gene, excluding the possibility of alternative splicing as the mechanism underlying the production of two SPT mRNAs. To examine a possibility that different initiation sites are used to generate the two SPT mRNAs, we determined the initiation sites of SPT gene transcription by means of primer extension and S1 nuclease protection analysis, as shown in Fig. 6. Two transcripts were detected on primer extension analysis: the larger transcript is an inducible RNA, which is detectable only after the administration of glucagon or insulin to the animals, whereas the smaller transcript seems to be constitutive. In this experiment, however, the relative amount of the larger and smaller transcripts

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**Fig. 4.** S1 nuclease protection assay with low resolution. cDNA fragment I, 5’-end-labeled at its Scfl site, was hybridized with 10 µg each of poly(A)+ RNAs prepared from the livers of normal rats (lane 3), glucagon-injected rats (lane 4), and DEHP-administered rats (lane 5, 2 days; lane 6, 3 days). S1 nuclease-resistant bands were analyzed by 7 M urea, 3% polyacrylamide gel electrophoresis and autoradiography. 5’-End-labeled HinII digest of pBR322 was applied to lane 1 as a size marker. Numbers to the left of lane 1 indicate the sizes (base pairs) of fragments. Untreated probe I was applied to lane 2. The electrophoresis was performed 2.5 times longer on the right than on the left. nt, nucleotides.

**Fig. 5.** Southern blot analysis of genomic DNA. High molecular weight genomic DNA (10 µg) extracted from rat liver was digested at 37 °C for 6 h with 100–200 units each of BamHI (lane 1), BglII (lane 2), EcoRI (lane 3), EcoRV (lane 4), KpnI (lane 5), HindIII (lane 6), PvuII (lane 7), PstI (lane 8), XhoI (lane 9), XbaI (lane 10), and Apal (lane 11). The digested samples were extracted with phenol, precipitated with ethanol, and then electrophoresed on a 0.7% agarose gel. Fragment C was used as a hybridization probe. kb, kilobase pairs.

The initiation sites of transcription for these mRNAs are shown in Fig. 8. SPTm mRNA is transcribed from nucleotide position -47 or -48 and codes for pSPTm, which consists of
FIG. 6. Determination of the transcriptional initiation sites of the SPT gene. Primer extension (lanes 1-3) and S1 nuclease mapping (lanes 4-6) were carried out using 10 μg each of poly(A)+ RNAs prepared from the livers of normal rats (lanes 1 and 4), glucagon-treated rats (lanes 2 and 5), and insulin-treated rats (lanes 3 and 6). Fragment J of genomic DNA used for S1 nuclease mapping and the synthetic oligonucleotide used for primer extension are presented in Fig. 1. Arrows and arrowheads indicate the specific bands detected on primer extension and S1 nuclease mapping, respectively. Lanes G, A, C, and T are the sequencing ladders of the genomic DNA obtained by the dideoxy chain termination method using the same oligonucleotide as a primer.

the N-terminal extension peptide of 22 amino acids and mature SPTm of 392 amino acids. SPTp mRNA, on the other hand, is transcribed from a position around +19 to +22, about 70 nucleotides downstream of the initiation site for SPTm mRNA transcription. Because in SPTp mRNA the ATG triplet at +67 to +69 is the first appearing Met codon and may be translated as the N-terminal Met, the resultant translation product (SPTp) is concluded to be completely the same as mature SPTm if no proteolytic cleavage or modification of N-terminal amino acid occurs after its biosynthesis. Both the SPTm and SPTp mRNAs have, in their 5′-untranslated regions, sequences complementary to the 3′-terminal sequence of 18S rRNA, as shown in Fig. 8. Because the direct interaction of eukaryotic mRNA with 18 S rRNA has been experimentally demonstrated (26), these sequences in eukaryotic mRNAs may contribute to the stabilization of the translational initiation complex. Furthermore, the sequence around the initial ATG codon in both mRNAs satisfies the most highly conserved requirement, i.e. purine at −3, for the efficient translation proposed by Kozak in her scanning model for translation (27, 28).

FIG. 7. Ribonuclease protection assay. At the top is the insert of a cDNA clone for pSPTm, pRspt 321, and cDNA fragments used for the preparation of anti-sense RNA probes. Narrow and wide boxes, hatched boxes, numbering of nucleotides, and numbers in parentheses are the same as in Fig. 1. 32P-Labeled RNA probes a–e were prepared from respective corresponding cDNA fragments as described under "Materials and Methods," and annealed at 50 °C (overnight) to 0.5 μg of poly(A)+ RNA prepared from the livers of glucagon-treated rats (lanes 4, 8, 12, 16, and 20) or 5 μg of poly(A)+ RNA prepared from the livers of normal rats (lanes 5, 9, 13, 17, and 21) in the presence of 10 μg of yeast tRNA. The hybridized RNA samples were then treated with ribonucleases A and T1, and undigested probes were analyzed by electrophoresis on a sequencing gel as described by Melton et al. (24). RNA size markers applied to lane 1 were SP6 transcripts from PAM19 linearized with BstNI (308 nucleotides) and MboII (151 nucleotides), and T7 transcript from PAM19 linearized with DdeI (249 nucleotides). Untreated probes a, b, c, d, and e were applied to lanes 2, 6, 10, 14, and 18, respectively. All samples applied to electrophoresis contained the same amount (10 μg) of yeast tRNA, and to lanes 3, 7, 11, 15, and 19 tRNA alone was applied. In this electrophoresis, the migration of probe a was less than expected as indicated by asterisk, probably due to highly complexed secondary structure of the probe caused by the high GC content. Normal migration of probe a was recently obtained using a sequencing gel containing 30% formamide.

DISCUSSION

We showed in this study that two kinds of SPT mRNA are generated from a single gene through differential initiation of transcription. The longer mRNA encodes the precursor for the mitochondrial serine:pyruvate aminotransferase and the shorter one should correspond to the peroxisomal aminotransferase, whose amino acid sequence should be identical with that of mature SPTm. Similar examples were reported in yeast, i.e. that the differential transcription of the same gene produced two types of mRNA encoding two proteins exhibiting different N-terminal amino acid sequences and different cellular or subcellular localization. In the case of the yeast
SUC2 gene encoding invertase (29, 30), the longer mRNA codes for the glycosylated, secreted form of the enzyme, whereas the shorter mRNA encodes the nonglycosylated, cytoplasmic form of the enzyme. In the cases of the HTS1 (31), FUM1 (32), VA91 (33), and LEU4 (34) genes of yeast, on the other hand, the longer mRNAs encode the mitochondrial enzymes and the shorter mRNAs the cytoplasmic enzymes. Serine:pyruvate aminotransferase is unique in that it is subcellularly localized in mitochondria and peroxisomes.

The precursor for the mitochondrial SPT encoded by SPTm mRNA, pSPTm, contains the N-terminal extension peptide of 22 amino acids, which is essential for the specific translocation of pSPTm into mitochondria. The extension peptide is removed from the precursor after the translocation into mitochondria. On the other hand, SPTp mRNA is transcribed from a position about 70 nucleotides downstream of the SPTm mRNA transcription site. Because SPTp mRNA lacks the first and second appearing Met codons of SPTm mRNA, the translated product does not contain the amino acid sequence corresponding to the N-terminal extension peptide of pSPTm. The product (SPTp) may be translocated into peroxisomes, instead of mitochondria, through the peroxisomal targeting signal(s) probably located in the enzyme molecule.

It is well established that the precursors of many mitochondrial proteins contain the N-terminal extension peptide which functions as mitochondrial targeting signal (35). The N-terminal extension peptide of pSPTm possesses a structural feature that there are four basic amino acids distributed among hydrophobic amino acids. This amphiphilic nature in the putative α-helical structure is characteristic of N-terminal extension peptides of many mitochondrial proteins (20). In contrast to the mitochondrial targeting signal, the peroxisomal targeting signal still remains to be clarified. Recently, it was reported (36, 37) that a tripeptide, with the sequence of Ser-(Lys, Arg, His)-Leu, located at or near the C terminus of peroxisomal proteins can act as a peroxisomal targeting signal. A similar sequence, Asn-Lys-Leu, is located at the C terminus and there is an internal Ser-Lys-Leu sequence in rat liver SPT (20). Whether or not these sequences in SPTp actually function as a peroxisomal targeting signal is under investigation.

As shown in this study, the amino acid sequence of the primary translation product for SPTp should be identical with the sequence of mature SPTm. This means that pSPTm has a peroxisomal targeting signal, probably present in the SPT molecule, in addition to the mitochondrial targeting signal included in the N-terminal extension peptide. A selective increase of SPT in mitochondria, but not in peroxisomes, on the administration of glucagon or insulin suggests that only the mitochondrial targeting signal functions in the pSPTm molecule, namely, the putative peroxisomal targeting signal is masked in the pSPTm molecule. Elucidation of the mechanism responsible for the expression of only the mitochondrial targeting signal in pSPTm remains to be performed.
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SPTm of rat liver is known to be induced selectively and markedly by glucagon administration (2, 38), through an increase of its mRNA content (5). Because SPTm mRNA begins to increase within 1 h after glucagon administration (6), this increase may be due to enhancement of transcription rather than a decrease in mRNA degradation. In addition to this hormone, we recently found (6) that insulin also causes a selective increase in SPTm mRNA through a mechanism different from that in the case of glucagon. Thus, the responses to hormone stimuli of the transcription of SPTm and SPTp mRNAs are quite different, although the two transcriptional initiation sites are located only about 70 nucleotides apart. The amount of the longer transcript (SPTm mRNA) is regulated by various hormones, whereas the shorter one (SPTp mRNA) seems to be constitutive. Detailed analysis of the 5'-flanking region of the SPT gene is required to elucidate the mechanism underlying the specific action of glucagon and insulin on the transcription of SPTm mRNA.

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