Structure and Organization of the Human S-Adenosylmethionine Decarboxylase Gene*

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Genomic clones for the S-adenosylmethionine (AdoMet) decarboxylase gene were isolated from a human chromosome 6 DNA library. In addition, polymerase chain reaction and specific primers were used to amplify fragments from chromosomal DNA covering exonic regions not found in the screening of DNA libraries with AdoMet decarboxylase cDNA. The gene encompasses at least 22 kilobases of chromosome 6 DNA and comprises nine exons and eight introns, in contrast to the corresponding rat gene that has only eight exons (Pulkka, A., Ihalainen, R., Aatsinki, J., and Pajunen, A. (1991) FEBS Lett. 291, 289-295). Exon-intron junctions in the human and rat AdoMet decarboxylase genes were in identical positions except that exons 6 and 7 of the human gene formed a single exon in the rat gene. Alu-like sequences are present in four introns and the 5'-flanking region of the human gene. The promoter region contains a TATA box adjacent to the cap site; in addition, DNA elements for binding of transcription factors AP-1, AP-2, CREB, SP-1, and multiple steroid receptors are present between position -3,158 and the transcription start site. Two AdoMet decarboxylase promoter-reporter gene constructs with 170 and 1,500 nucleotides of the 5'-flanking DNA were used in transient expression studies. AdoMet decarboxylase promoter was capable of driving reporter gene expression, but it was less active than the murine ornithine decarboxylase promoter. There are at least three potential polyadenylation signals at the 3'-end of the gene, and utilization of the first two results in the formation of the 2.0- and 3.6-kilobase AdoMet decarboxylase mRNA species present in human tissues and cell lines. AdoMet decarboxylase gene-related sequences were also present in human chromosomes 6 and X (10). The presence of multiple AdoMet decarboxylase gene-related loci was also determined for rat and mouse genome, and nucleotide sequences for intronless and intron-containing rat AdoMet decarboxylase genomic DNAs have been published (7, 11, 12). The results of this work show that an active AdoMet decarboxylase gene is localized on human chromosome 6 and that the locus on human chromosome X appears to represent a processed AdoMet decarboxylase pseudogene.

S-Adenosylmethionine (AdoMet)\textsuperscript{1} decarboxylase (EC 4.1.1.50) is one of the key enzymes in polyamine biosynthesis, and the product of the catalytic reaction, decarboxylated AdoMet, serves as an aminopropyl donor in the biosynthesis of spermidine and spermine (1-4). The concentration of decarboxylated AdoMet in mammalian cells is very low under physiological conditions, suggesting that its availability may actually control the rate of polyamine formation (1, 2). Similar to the other decarboxylase in the biosynthetic pathway of polyamines, ornithine decarboxylase, AdoMet decarboxylase protein has a rapid turn-over rate, represents a minor component among cellular proteins, and is highly regulated by a variety of physiological stimuli (1-6). Cloning and sequencing of human and rat AdoMet decarboxylase cDNAs have revealed that the mammalian enzyme is synthesized as a M\textsubscript{r} 38,000 proenzyme, which is autocatalytically cleaved to form two subunits with M\textsubscript{r} of 31,000 and 7,000 (7). Both subunits appear to be necessary for the catalytic activity, whereas the uncleaved proenzyme is inactive (8). The enzyme protein sequence is highly conserved among different mammalian species, with the human, rat, and bovine proteins exhibiting amino acid sequence identity of about 90% (7, 9).

The present work was undertaken to characterize the structure of the human gene encoding AdoMet decarboxylase and to define possible regulatory elements in its promoter. Previous studies have indicated that the human genome contains at least two loci recognizable by hybridization to a rat AdoMet decarboxylase cDNA, which were mapped to human chromosomes 6 and X (10). The presence of multiple AdoMet decarboxylase gene-related loci was also determined for rat and mouse genome, and nucleotide sequences for intronless and intron-containing rat AdoMet decarboxylase genomic DNAs have been published (7, 11, 12). The results of this work show that an active AdoMet decarboxylase gene is localized on human chromosome 6 and that the locus on human chromosome X appears to represent a processed AdoMet decarboxylase pseudogene.

\section*{MATERIALS AND METHODS}

\textit{Chemicals, Isotopes, and Enzymes—}\textsuperscript{pGEM-3Z was purchased from Promega (Madison, WI). DNA libraries specific for human chromosomes 6 and X, pRSVcat, pSV\textsuperscript{+}cat, HepG2 human hepatoma cells, and NIH/3T3 mouse cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). pPL\textsuperscript{+}cat was prepared by inserting a polylinker containing multiple restriction enzyme sites in front of the chlamydomenical acetyltransferase gene of pSV\textsuperscript{+}cat (13). pSV-\textsuperscript{+}galactosidase (pSV-\textsuperscript{+}gal) control plasmid was from Promega (Madison, WI). Oligonucleotides were synthesized using a Pharmacia gene assembler (Pharmacia LKB Biotechnology Inc.). O-Nitrophenyl-\textsuperscript{+}galactosidase was from Sigma. Escherichia coli strain DH\textsubscript{5a} competent cells were purchased from GIBCO-BRL.}\textsuperscript{18915-18923,1992}

\textsuperscript{1} The abbreviations used are: AdoMet, S-adenosylmethionine; kb, kilobase(s); Na\textsubscript{2}DODSO\textsubscript{4}, sodium dodecyl sulfate; nt, nucleotide(s).
ATP (3.000 Ci/mmol), [α-32P]dCTP (3.000 Ci/mmol), and [3H]acetyl-CoA (0.2 Ci/mmol) were from Du Pont-New England Nuclear. [35S]dATP (800 Ci/mmol) was purchased from Amer sham Corp.

Restriction endonucleases, calf intestinal alkaline phosphatase, and T4 DNA ligase were from Boehringer Mannheim (Mannheim, Germany). DNA polymerase I (Klenow fragment) was purchased from Pharmacia. T4 DNA ligase was from New England Biolabs (Beverly, MA). Taq DNA polymerase was obtained from Perkin-Elmer/Cetus. Avian myeloblastosis virus reverse transcriptase and RNasin were from Promega.

Identification of the DNA fragments was carried out using Gene Clean II from BBIOL (La Jolla, CA) according to the manufacturer's instructions. Reagents for labeling of DNA samples by the random priming method were purchased from Boehringer Mannheim. Sequencing of double-stranded DNA was performed using the dideoxy chain-termination method (14) with reagents obtained from either U. S. Biochemicals or Pharmacia.

**Screening of Genomic DNA Libraries**—Three human genomic DNA libraries were initially screened. Two of these contained chromosome 6-specific sequences (ATCC nos. 57570 (HindIII-digested) and 57721 (EcoRI-digested)), and the third one was specific for the X chromosome (no. 57750 (a partial Sau3A digest)). [32P]-Labeled pSAM1 DNA (7) was used as hybridization probe in the screening of at least two genomic equivalents of each library, and all DNA-DNA hybridizations were carried out using standard techniques (15, 16). Screening of the library 57570 yielded initially six positive phage clones and, after subcloning, three unique clones which were used for further characterization. The library 57750 gave nine positive signals when screened with [32P]-labeled pSAM1 DNA. Screening of the library 57721 did not yield any phage clones that remained positive throughout plaque purification.

Sequences missing in the clones derived from chromosome 6-specific libraries were isolated either by amplification of chromosomal DNA by polymerase chain reaction (see below) or by screening a human total genomic DNA library (Clontech, Palo Alto, CA). Sequences corresponding to the first intron of the AdoMet decarboxylase gene were isolated from the genomic library. Hybridization probes for screening were synthesized in the text, and for sequencing, three unique primers from the library were used for further characterization. The library 57750 gave nine positive signals when screened with [32P]-labeled pSAM1 DNA. Screening of the library 57721 did not yield any phage clones that remained positive throughout plaque purification.

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**Reverse Transcription-Polymerase Chain Reaction**—Reverse transcriptase-catalyzed synthesis of cDNA and amplification of cDNA by polymerase chain reaction with Taq DNA polymerase was conducted in a total volume of 100 μl and 200 ng of cDNA. Reactions were performed with 1× Taq polymerase buffer (200 mM Tris-HCl, pH 8.3, 1.5 mM MgCl2, 50 mM KCl, 0.01% (w/v) gelatin, 200 μM each of the four dNTPs, 1 μM each primer, and 2 units of Taq DNA polymerase. The reaction mixtures were incubated at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. After each of the four dNTPs, and 1 μM each primer), and RNasin (40 units), avian myeloblastosis virus reverse transcriptase (35 units), corresponding to the

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to a KpnI restriction site). The 5694 DNA fragment was cloned into the Smal site of pPLcat vector, whereas 7662 DNA fragment was inserted between the KpnI and HindIII sites of pPLcat. The orientation of DNA fragments in the two constructs was confirmed by DNA sequencing.

Transfection of Cultured Cells—HePG2 and NIH/3T3 cells were cultured in a medium containing Dulbecco's modified Eagle's medium supplemented with 10% calf serum. The cells were transfected with 10 μg of reporter gene plasmid DNA using the calcium phosphate coprecipitation method as described in (16). Five μg of pSV-β-galactosidase plasmid was cotransfected with the CAT constructs to serve as an internal control for transfection efficiency. All transfection experiments were performed using duplicate dishes. After a 16-h incubation with the precipitate, the cells were washed with phosphate-buffered saline and fresh medium was added. Cells were harvested 24 h after the medium change. Chloramphenicol acetyltransferase assays were carried out as described by Neumann et al. (22) and Eastman (23). β-Galactosidase activity was determined according to the method of Rosenthal (24). CAT activity was divided by β-galactosidase activity to normalize the data for transfection efficiency.

Other Procedures—Isolation of phase DNA was carried out as described (25). Subcloning of DNA fragments into plasmid vectors were performed using standard techniques (15, 16). Poly(A)-containing RNA was isolated by chromatography on oligo(dT)-cellulose (26). Protein concentration was determined by the dye-binding method (Bio-Rad) according to the manufacturer's instructions.

RESULTS

Organization of the AdoMet Decarboxylase Gene—Structural analysis of the three clones isolated from human chromosome 6-specific library and two clones amplified by polymerase chain reaction from chromosomal DNA revealed that the human AdoMet decarboxylase gene covers at least 22 kilobases (kb) of chromosome 6 DNA. The gene comprises nine exons and eight introns (Fig. 1, Table I). The three clones isolated from the chromosome 6 library contained exons 1, 5, 6, 7, 8, and 9 (Fig. 1). Approximately 3 kb of 5' flanking DNA, exon 1, and about 2.3 kb of intron 1 were included in clone chr6/701-121, clone chr6/701-01 contained 681 nt of intron IV, exons 5–8, and 156 nt of intron VIII, whereas 257 nt of intron VIII, exon 9, and about 2 kb of 3' flanking DNA were present in clone chr6/701-15 (Fig. 1).

Since these clones isolated from chromosome 6-specific library did not have overlapping sequences, as the library was prepared from a complete HindIII digest, amplification of intron VIII sequences from human genomic DNA was used to verify that a unique HindIII site joins clones chr6/701-01 and chr6/701-15 together (data not shown). None of the three chromosome 6-specific clones contained sequences encompassing AdoMet decarboxylase cDNA region from nt 431 to 747, which includes exons 2, 3, and 4. The EcoRI-digested chromosome 6-specific library was also devoid of these sequences. The clone p117119 was isolated from human chromosomal DNA using polymerase chain reaction. The primers were designed to cover most of the cDNA between nt 431 and 747. Spacing between the ends of each primer pair was only a few nt of cDNA sequence. For example, 3'-end of SAM117 and 5'-end of SAM118 were separated by only seven nucleotides of exonic sequence. This pair covered the 5'-end of the missing DNA fragment. The second pair (SAM115 and SAM116) had two nucleotides between the ends and was present close to the 3'-end of the missing piece. SAM119, located to the beginning of intron IV, was paired for amplification with either SAM115 or SAM117. Amplification with SAM115 and 116, SAM115 and 119, and SAM117 and 118 gave a single DNA fragment of 1.4–1.5 kb in length. The size of fragment SAM117119 was about 3.0 kb. When hybridized to 32P-labeled pSAMh1 DNA, only fragments SAM115119 and SAM117119 gave positive signals, as they contained enough exonic DNA sequence to permit a stable hybrid formation with AdoMet decarboxylase cDNA. Most of the sequence in fragments SAM115116 and SAM117118 was intronic and did not hybridize to the cDNA probe (data not shown). The clones isolated this way extended from the 3'-end of exon 2 to the 5'-end of intron IV, with 58 nt of the fourth intron being included (Fig. 1).

Despite screening of multiple human genomic DNA libraries, we were unsuccessful in isolating AdoMet decarboxylase gene clones covering the junction between the 3'-end of intron I and the 5'-end of exon 2. To isolate this region, we took advantage of the highly conserved nature of the second monomeric sequence of Alu repeats, and carried out amplification of human chromosomal DNA with primers containing this Alu repeat sequence (19) and the 3'-end of exon 2. Using this approach, a fragment of ~900 nt in length was amplified from chromosomal DNA, and it spanned the boundary between intron I and exon 2 (p517134 in Fig. 1).

Exons 5, 6, 7, and 8 were included in clone chr6/701-01. To verify the organization of this region, human chromosomal DNA was amplified with intron-specific primers which were positioned 62 nt upstream from the 5'-end of exon 6 and 67 nt downstream from the 3'-end of exon 7. The amplified fragment was 0.66 kb in length and hybridized to human AdoMet decarboxylase cDNA. Nucleotide sequence of this fragment was in agreement with that in clone chr6/701-01, which confirmed that there is indeed a 294-nt-long intervening sequence between exons 6 and 7. Organization of the human AdoMet decarboxylase gene in this region was studied in detail, as a previous report on the rat gene had shown a different organization (12).

**Fig. 1.** Organization of the human AdoMet decarboxylase gene. Arrangement of exons (boxes and Roman numerals) and introns (solid lines) are depicted. The protein-coding region is identified by the cross-hatched boxes, and the 5'- and 3'-untranslated regions are shown as open boxes. Positions of the λ clones and clones constructed using polymerase chain reaction are defined by lines with arrowheads. Location of HindIII (H), PatI (P), and EcoRI (E) restriction enzyme sites in the gene are also shown. 5'-UTR, 5'-leader sequence of AdoMet decarboxylase mRNA; poly(A)' and poly(A)" refer to the two major polyadenylation/termination sequences.
introns, respectively. The size of intron \(I\) represents a minimum estimate, which was determined by restriction enzyme analysis and Southern blotting of three clones isolated from a human DNA library.

Identification of Transcription Start Site—Primer extension with RNA samples from HepG2 and HELa cell lines was used to determine the start site for AdoMet decarboxylase mRNA transcription. The same G residue was identified with the two RNA samples (Fig. 2). These results showed that the 5’-leader of the human AdoMet decarboxylase mRNA is 320 nt long, i.e. it extends 72 bases beyond the 5’-end of AdoMet decarboxylase cDNA, SAM1, which has been previously sequenced (7). The cap site for the human mRNA is localized to the same nt as that in the rat mRNA, although the 5’-untranslated region of the rat mRNA is 5 nt longer than that of the human (see below).

Definition of the 3' Termini for AdoMet Decarboxylase mRNA Species—There are two AdoMet decarboxylase mRNAs with molecular sizes of about 2.0 and 3.6 kb in human tissues and cell lines (7). The first polyadenylation signal giving rise to the shorter mRNA (site \(I\), Fig. 3) was already included in the pSAM1 sequence (7), and it begins 556 nt after the end of the AdoMet decarboxylase protein-coding sequence. Nucleotide sequence downstream of the first polyadenylation signal revealed the presence of at least two additional potential polyadenylation/termination signals (AA-TAAA and AAATAAG, sites \(II\) and \(III\) in Fig. 3, respectively). Reverse transcription-polymerase chain reaction amplification with primers bracketing site \(II\) (SAM148 is immediately 5’ to site \(II\) and SAM149 ~80 nt downstream of this site) was used to define the 3’-end of the longer mRNA. The results indicated that site \(II\) is the predominant polyadenylation/termination signal for the longer mRNA; however, even site \(III\) appears to be used in vivo (Fig. 3, inset). The distance between sites \(I\) and \(II\) is 1,534 nt, which figure is close to the size difference of about 1,600 nt measured by RNA blotting for the two AdoMet decarboxylase mRNA species (7).

Nucleotide Sequence of the Human AdoMet Decarboxylase Gene—Nucleotide sequence determined for the protein-coding region of the chromosomal gene was identical to that previously reported for AdoMet decarboxylase cDNA (7) with two mismatches. There is a C instead of a G at position 757 (numbering from the cap site), which changes the codon Gly\(^{146}\) to Ala\(^{146}\). The nucleotide at position 1575 (in the 3’-untranslated region) is a C in the genomic sequence rather than a G in the cDNA. In contrast to the report of Stanley et al. (8), our genomic sequence for the codon Ala\(^{97}\) was the same as that in AdoMet decarboxylase cDNA (7).

Fig. 4 shows the sequence of the entire human AdoMet decarboxylase gene, with the exception of gaps in introns 1, 3, and 4. There are Alu-like sequences in four introns and in the 5’-flanking region of the gene. The entire 5’-leader sequence is contained within exon 1 that also includes 110 nt of the protein-coding sequence. The lengths of the exons, sizes of introns, and sequences for the intron/exon junctions are listed in Table I. The general consensus (27) for the 5’-splice recognition sequence (GTAAGT) is present in most of in-
tronic sequences of the AdoMet decarboxylase gene. There are CTTTTT or ATTTTT hexamers present upstream from the 3'-acceptor site in all introns except for the fourth one. These hexamers are usually 4-11 nt from the 3'-acceptor AG dinucleotide (Fig. 4).

Table II lists locations of potential transcription factor-binding sites in the 5'-flanking DNA of the AdoMet decarboxylase gene extending from nt -3,158 to the transcription start site. The promoter region contains a TATA box sequence, several DNA motifs for AP-1 and SP-1 binding, a potential cAMP response element, and a number of half-sites for interaction with glucocorticoid, androgen, and estrogen receptors (Table II). Whether these motifs actually function in vivo as cis-acting elements for transactivating proteins remains to be investigated. It is worth mentioning, however, that AdoMet decarboxylase activity, at least in rodent tissues, is known to be induced by a number of factors mediating their actions by mechanisms involving the above-mentioned DNA motifs (5, 6).

A pyrimidine-rich initiator (Inr (28-31)) overlapping the transcription start site is present in the human AdoMet decarboxylase gene. The Inr sequence YYYYYGYYYYY, where Y is a pyrimidine and G the nucleotide for transcription initiation, is also present in the rat gene at the same location (12). Inr and Inr-like elements have been previously described for adenovirus major late, human immunodeficiency virus-1, and terminal deoxynucleotidyl transferase promoters (28-30). A novel transcription factor (TFII-I) has recently been shown to bind to the Inr element and to interact co-operatively with the basal promoter transcription factor USF (31). Elucidation of the function of this sequence in the AdoMet decarboxylase promoter will be of interest, as it may play a role in its ubiquitous activity.

Southern Blotting of Human DNA—Use of AdoMet decarboxylase cDNA as the hybridization probe in Southern blotting revealed the presence of a number of hybridizable fragments (Fig. 5), a finding similar to that reported in blotting of human DNA with a rat AdoMet decarboxylase cDNA (10). Since the complete sequence of the long first intron is not currently known, it is not possible to decide with absolute certainty the origin of the hybridizable fragments. On the basis of DNA sequence data (Fig. 4) and chromosomal localization by Radford et al. (10), fragments of 7.2, 6.0, 5.4, and 2.2 kb in length of the PstI-digested sample appear to originate from the active AdoMet decarboxylase gene residing on chromosome 6. Of these fragments, the 5.4-kb band most likely includes the 5'-end and the flanking region of the gene, whereas the 2.2-kb band encompasses exons 3 and 4. The 2.9-kb-long PstI fragment has been previously reported to map to the X chromosome (10). A fragment of identical length was isolated from the X chromosome-specific library, its nucleotide sequence partially determined, and found to represent a processed AdoMet decarboxylase pseudogene. With primers specific for AdoMet decarboxylase cDNA, we have sequenced exon/intron boundaries for six potential cases and have not found a single intervening sequence.2

Comparison of Human and Rat AdoMet Decarboxylase Gene Organizations—Organizations of human and rat AdoMet decarboxylase genes are depicted in Fig. 6. Even though the amino acid and nucleotide sequences of human and rat AdoMet decarboxylase protein and mRNAs are highly conserved, given in Table I. The polyadenylation/termination signals utilized for generation of the two AdoMet decarboxylase mRNA species are identified by underlined and italicized letters. Abbreviation: IVS, intervening sequence.

Fig. 4. Nucleotide sequence of the human AdoMet decarboxylase gene. Exonic and intronic sequences are specified by capital and lowercase letters, respectively. The locations of the five Alu repeats are underlined. The gaps in sequence are indicated by stars. Numbering is given only for the exons, with the transcription start site being number 1. The lengths of the intronic sequences are

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Table II
Location of potential transcription factor-binding sites in the 5'-flanking region of the human AdoMet decarboxylase gene
Sequences for the DNA motifs are taken from (40).

<table>
<thead>
<tr>
<th>Transcription factor (consensus sequence)</th>
<th>Sequence in the gene</th>
<th>Location in the 5'-flanking region*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>TGAGTAA</td>
<td>(-2736, -2466)</td>
</tr>
<tr>
<td></td>
<td>TGGGTCA</td>
<td>(-66)</td>
</tr>
<tr>
<td>TFIID/TBP (TATAAA)</td>
<td>TATAAG</td>
<td>(-29)</td>
</tr>
<tr>
<td>AP-2</td>
<td>GCCTGGGC (rev)</td>
<td>(-1455)</td>
</tr>
<tr>
<td></td>
<td>CCCCTGGG</td>
<td>(-545)</td>
</tr>
<tr>
<td></td>
<td>CCCCCGGGCC</td>
<td>(-160)</td>
</tr>
<tr>
<td></td>
<td>CGCCAGGC</td>
<td>(-149)</td>
</tr>
<tr>
<td></td>
<td>GGAGGAGGC</td>
<td>(-43)</td>
</tr>
<tr>
<td>CREB/ATF (TGAGGG&lt;sub&gt;T&lt;/sub&gt;/G&lt;sub&gt;A&lt;/sub&gt;)</td>
<td>TGACATCA</td>
<td>(-1257)</td>
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<tr>
<td>CTF/NF-1 (T&lt;sub&gt;C&lt;/sub&gt;G&lt;sub&gt;A&lt;/sub&gt;/G&lt;sub&gt;N&lt;/sub&gt;GGCCAA)</td>
<td>AGCCAAT</td>
<td>(-2345)</td>
</tr>
<tr>
<td></td>
<td>ATTTGGGC (rev)</td>
<td>(-1576)</td>
</tr>
<tr>
<td></td>
<td>GGCCAGAG</td>
<td>(-459)</td>
</tr>
<tr>
<td></td>
<td>TGGAGGTTGGGACGA</td>
<td>(-80)</td>
</tr>
<tr>
<td>CBP (CCAAT)</td>
<td>CCAAT</td>
<td>(-2077, -1739)</td>
</tr>
<tr>
<td>GR/AR (TGTTCT)</td>
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<tr>
<td></td>
<td>CGTTCT</td>
<td>(-1339)</td>
</tr>
<tr>
<td></td>
<td>TGTTT</td>
<td>(-990)</td>
</tr>
<tr>
<td></td>
<td>TGTTCT</td>
<td>(-54)</td>
</tr>
<tr>
<td>SP-1&lt;sup&gt;a&lt;/sup&gt; (G&lt;sub&gt;T&lt;/sub&gt;G&lt;sub&gt;A&lt;/sub&gt;/GCCG&lt;sub&gt;T&lt;/sub&gt;G&lt;sub&gt;A&lt;/sub&gt;/G&lt;sub&gt;A&lt;/sub&gt;/G&lt;sub&gt;A&lt;/sub&gt;/G&lt;sub&gt;T&lt;/sub&gt;)</td>
<td>CCGGCC (rev)</td>
<td>(-197)</td>
</tr>
<tr>
<td></td>
<td>GGCGGG</td>
<td>(-24)</td>
</tr>
<tr>
<td>ER&lt;sup&gt;a&lt;/sup&gt; (AGGTCA)</td>
<td>GGTCGA</td>
<td>(-2339, -2910, -2492, -2300, -1639)</td>
</tr>
</tbody>
</table>

* The number refers to the first nucleotide of the sequence in question.

<sup>a</sup> Abbreviations: AP-1, fos/jun protein complex; TFIID/TBP, TATA-binding protein; CREB/ATF, cAMP responsive element-binding protein; CBP, caat box-binding protein; GR/AR, glucocorticoid/androgen receptor; SP-1, GC box-binding protein; ER estrogen receptor; rev, the DNA motif is in the opposite strand.

* Only half-sites for the steroid receptor-binding elements are depicted.

served (7), exon/intron organizations of the AdoMet decarboxylase gene in these two species showed an intriguing difference, in that exons 6 and 7 of the human gene formed a single exon 6 in the rat gene (Fig. 6). As mentioned previously, the human gene organization for exon 6, intron VI, and exon 7 was verified by amplifying this region from chromosomal DNA and sequencing the amplified fragment. In all other cases, exon/intron junctions of the human and rat genes were in identical locations. In addition to the previously reported similarity between human and rat AdoMet decarboxylase mRNAs, including the first 250 nt of the 5'-noncoding sequence, the distal 5'-untranslated regions of the two mRNAs were found to be extremely well conserved (Fig. 7). The 5'-leader sequence of the human mRNA was 320 nt in length, as opposed to a length of 325 nt of the rat gene. The proximal promoters of the two genes were highly similar up to nt -65 (in the human gene), after which there was an abrupt dissimilarity between the promoter sequences (Fig. 7).

**Functional Analysis of the AdoMet Decarboxylase Promoter—** Nucleotide sequence for the 5'-flanking region of the human AdoMet decarboxylase gene was determined in this work up to base -3,158 from the cap site (Fig. 4). To verify that this region indeed exhibited promoter activity, two pieces of 5'-flanking DNA were employed to devise Cat reporter gene constructs. The distal AdoMet decarboxylase promoter construct (p5604cat) included the sequence from -1,505 to +112, whereas the proximal one (p7662cat) contained the sequence from -173 to +103. In transient expression studies with HepG2 cells, p7662cat construct yielded enzyme activities that were about one-fourth of those measured for a Rous sarcoma virus promoter-driven reporter gene constructs. The distal AdoMet decarboxylase promoter construct (p5604cat) included the sequence from -1,505 to +112, whereas the proximal one (p7662cat) contained the sequence from -173 to +103. In transient expression studies with HepG2 cells, p7662cat construct yielded enzyme activities that were about one-fourth of those measured for a Rous sarcoma virus promoter-driven reporter construct (Fig. 8). In these experiments with HepG2 cells, the longer promoter construct (p5604cat) had less than 50% of the activity of the proximal one (Fig. 8). Whether this implies that there are silencing elements in the distal part of the AdoMet decarboxylase promoter remains to be elucidated. Transient expression...
studies with the same promoter constructs were also carried out using NIH/3T3 murine fibroblasts as recipient cells. In comparison to the murine ornithine decarboxylase promoter-driven construct (pODCcat (13)), p5604cat and p7662cat constructs had weak but clearly measurable promoter activity, which ranged from 10 to 35% of that determined for pODCcat (data not shown). As reported previously, however, the murine ornithine decarboxylase promoter (extending from −1,658 to +14) was strong in NIH/3T3 cells and yielded activities that were 50–65% of those for the Rous sarcoma virus promoter.

**DISCUSSION**

In the present work, we have elucidated structural organization for the human AdoMet decarboxylase gene and determined much of the nucleotide sequence of this gene encompassing over 20 kb of human chromosome 6 DNA. In addition to the active gene locus on chromosome 6, AdoMet decarboxylase gene-related sequences have been found to be present on the human X chromosome (10). Partial sequencing of this DNA indicated that the X-linked locus contains a processed AdoMet decarboxylase pseudogene. The presence of a similar pseudogene has also been reported for the rat genome (11).

The human AdoMet decarboxylase gene comprises nine exons and eight introns. It is somewhat surprising that its organization is different from that of the active rat gene (12), which has only eight exons and seven introns, as the human and rat AdoMet decarboxylase mRNAs and the encoded enzyme proteins are remarkably well conserved (7). Another peculiar feature between the human and rat genes is the total divergence of their 5'-flanking sequences upstream from nucleotide −65 of the human gene. This is particularly perplexing in view of the fact that the AdoMet decarboxylase gene codes for a housekeeping enzyme, whose regulation is not expected to be controlled by vastly dissimilar factors among different species. In this context, it is of interest to note that there are repetitive elements both within and around the human and the rat gene (12) (Alu, B1, and B2 repeats, respectively), which may have some bearing on the evolution of their structural differences. Since the species differences were unexpected, we verified two issues pertaining to the structure of the human gene. First, the region encompassing exons 6 and 7 was amplified directly from human chromosomal DNA, and its organization was determined to be identical with that of clone chr6/701-1 isolated from chromosome 6-specific DNA library. And second, the human AdoMet decarboxylase promoter was shown to be active in both human and murine cells, in that it was capable of driving expression of the bacterial chloramphenicol acetyltransferase gene used as a reporter.

When the cloning and sequencing of the human and rat AdoMet decarboxylase mRNAs were carried out, it was found that some rat cDNA clones lacked a 21-nt-long piece corresponding to nucleotides 645–665 (7). At that time, we did not know whether the short rat cDNAs were cloning artifacts or actually derived from transcribed AdoMet decarboxylase mRNA species. In both the human (Fig. 4) and the rat gene (12), this 21-nt-long sequence forms the beginning of exon 4 and has an AG dinucleotide at the 3'-end. Therefore, it is not unlikely that an AdoMet decarboxylase mRNA missing this piece is formed through alternative splicing of the primary transcript, although a formal proof for this postulate is still lacking. As this deletion maintains the reading frame of the encoded protein, it is an intriguing possibility that there are peculiar features between the human and rat genes.

![Figure 5](image-url)  
**Fig. 5.** Analysis of the human AdoMet decarboxylase gene by Southern blotting. High molecular weight human DNA (10 µg/lane) was digested with HindIII (H), Sall (S), PstI (P), and EcoR1 (E) restriction enzymes and size-fractionated by electrophoresis on 0.8% agarose gel. After electrophoresis, DNA was transferred to Duralose membrane, immobilized by UV light, and hybridized with 32P-labeled pSAM1 DNA, as described under “Materials and Methods.” The fragment corresponding to the AdoMet decarboxylase pseudogene residing on X chromosome is indicated by an arrowhead.

![Figure 6](image-url)  
**Fig. 6.** Comparison of the rat and human AdoMet decarboxylase gene organizations. The exons are depicted by boxes, with the closed and open boxes referring to the protein-coding region and untranslated sequences, respectively. The lengths of the exons are shown above the boxes and those of the introns below the solid lines. The origin of the 3'- untranslated regions for the short and long AdoMet decarboxylase mRNAs is designated by lines with arrowheads. Exons 6 and 7 of the human AdoMet decarboxylase gene form a single exon in the rat gene, as depicted by the two lines connecting the corresponding regions.
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**Fig. 7. Comparison of proximal promoters of the human and rat AdoMet decarboxylase genes and 5′-untranslated regions and the corresponding mRNAs.** The BESTFIT program (41) identified 93% sequence identity between the human (upper row) and rat (lower row) sequences, with four gaps (indicated by dots), when the comparison was made from the transcription start site to nt -65 of the human gene. The human and rat 5′-untranslated regions were shown to exhibit 96% nucleotide sequence identity using the same program.

![Diagram](image)

**Fig. 8. Transient expression of two AdoMet decarboxylase promoter-reporter gene constructs in HepG2 cells.** Plasmids p5604cat and p7662cat contained 1617 nt (−1505 to +112) and 276 nt (−173 to +103) of the human AdoMet decarboxylase promoter, respectively. The two promoter sequences were inserted in front of a promoterless chloramphenicol acetyltransferase gene vector (pPLcat). Expression of pRSVcat, containing the Rous sarcoma virus promoter, was used in this study as a reference for AdoMet decarboxylase-promoter-reporter gene constructs in HepG2 cells. Transfections with the pSV-o-galactosidase reporter gene construct corresponded to those regions previously identified by Kashwagi et al. (38) by comparing yeast and human AdoMet decarboxylase protein sequences. The 5′-untranslated regions of human and yeast AdoMet decarboxylase mRNAs exhibited no significant sequence homology; however, the 3′-untranslated regions contain a stretch of 60 nt with 79% sequence identity, which ends 21 nt prior to the first polyadenylation signal AATTAAA in the human mRNA.3 The significance of this nucleotide sequence homology remains to be elucidated.

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**REFERENCES**

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