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# Exon/Intron Organization and Transcription Units of the Human Acyl-CoA Synthetase 4 Gene

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**Acyl-CoA synthetase 4 (ACS4) is an arachidonate-preferring isozyme of ACS family predominantly expressed in steroidogenic tissues. Isolation and characterization of genomic clones encoding human ACS4 revealed that the genomic organization of the gene. The human ACS4 gene spans approximately 90 kb and consists of 16 exons. Sequence inspection of the 5′-flanking region revealed potential DNA elements including GATAs, p300, AP-4, SRY, CREB and MyoD. A minimal promoter region required for the expression of ACS4 in HeLa S3 cells was determined. The human ACS4 gene was mapped between the STS markers, WI-17685 and CHLC.GATA81B07 on Xq22–23 region.**

***Key Words:*** **acyl-CoA synthetase; arachidonate; fatty acid metabolism; genomic organization.**

Production of acyl-CoA, by acyl-CoA synthetase (ACS, EC6.2.1.3), is an essential reaction in mammalian cells. In previous studies, we have characterized five rat ACSs (ACS1–5) with different fatty acid specificity and tissue distribution. ACS3 (1) and ACS4 (2) are arachidonate-preferring isozymes closely related in structure, whereas ACS1 (3), ACS2 (4), and ACS5 (5) are classified into a subfamily of structurally resembling isozymes with similar fatty acid preference.

ACS4 is expressed in steroidogenic tissues including the adrenal gland, ovary, and testis (2), and utilizes arachidonate and eicosapentaenoate most preferentially. In the previous study, we showed that the expression ACS4 in steroidogenic tissues is posttranscriptionally induced by ACTH and suppressed by glucocorticoid. Furthermore, the levels of ACS4 mRNA and protein in steroidogenic cells were induced by dibutyryl cAMP, forskolin, and arachidonate (6).

The human ACS4 gene is mapped on chromosome Xq close to the $\alpha$5 chain of type IV collagen gene that is related to X-linked Alport syndrome. Piccini *et al.* showed that the ACS4 gene is deleted in patients with Alport syndrome, elliptocytosis and mental retardation (7). Recently we have shown that female mice heterozygous for ACS4 deficiency become pregnant less frequently and produce small litters with extremely low transmission of the disrupted alleles (8). Striking morphological changes including extremely enlarged uteri and lumina filled with numerous proliferative cysts of various sizes were shown in ACS4± females. Furthermore, marked accumulation of prostaglandins was seen in the uterus of the heterozygous females. These results indicate that ACS4 mediates female fertility and uterine prostaglandin production.

To further clarify the mechanism regulating ACS4 gene expression, we have cloned and characterized the gene encoding human ACS4. Here we describe the exon/intron organization and transcription units of the human ACS4 gene.

## EXPERIMENTAL PROCEDURES

*Materials and general procedures.* Unless otherwise indicated, all restriction and DNA-modifying enzymes were from Takara Shuzo Corp. (Kyoto, Japan). [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol) was from Amersham Pharmacia Biotech Corp. Oligonucleotides were synthesized with an automated DNA synthesizer (Applied Biosystems Inc., Model 381A).

Standard molecular biology techniques were performed essentially as described by Sambrook and Russell (9). Genomic clones were subcloned into pBluescript vectors for sequencing and PCR analysis. Nucleotide sequence was determined by the dideoxy chain-termination method with a Dye-Terminator Cycle Sequencing Ready Reaction FS kit (PE Biosystems) and a DNA sequencer (model 310.

Abbreviations used: ACS, acyl-CoA synthetase; BAC, bacterial artificial chromosome; STS, sequence-tagged site; UTR, untranslated region; YAC, yeast artificial chromosome.

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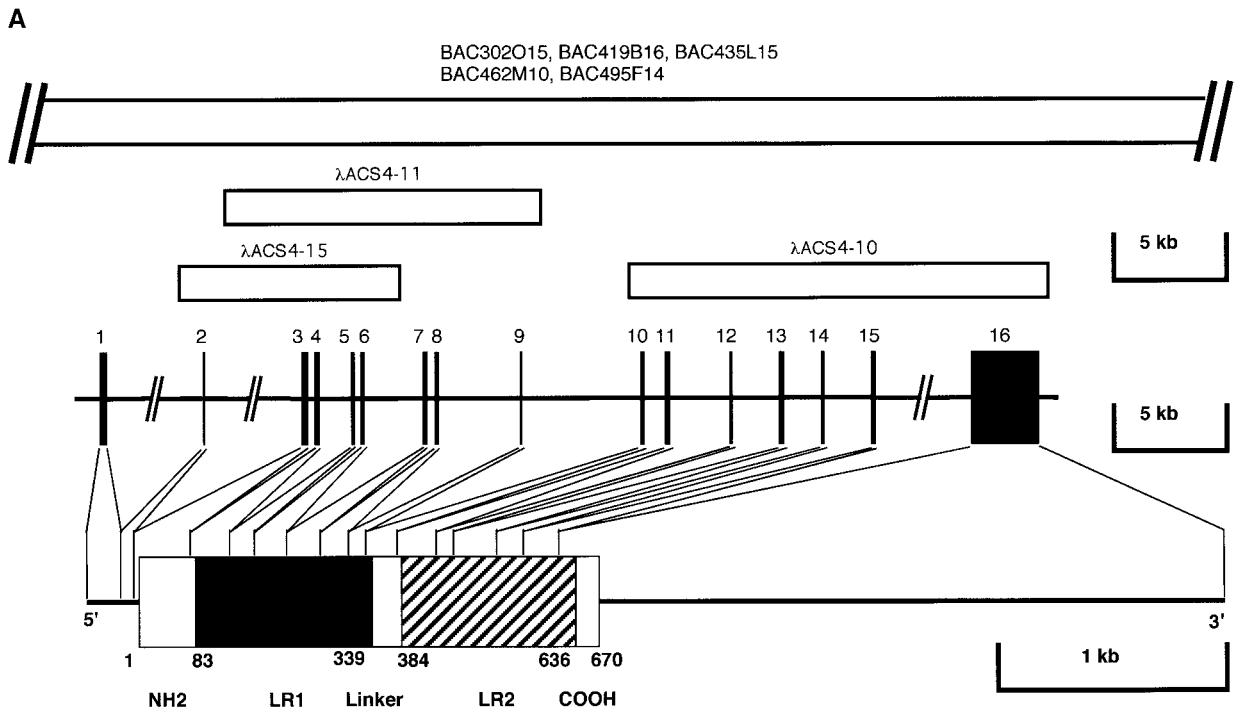


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hACS1     1 -------MQAHEL-FRYFRMPELVDFRQY-VRTLPTNTLMGFGAFAAL--TTF-WYA-TRPKPLKPPCDLSMQSVEVAGSGGARRSALLD    77
hACS2     1 -------MQTQEI-LRILRLPELGDLEQF-FRSLSATTLVSMGALAAI--LAY-WFT-QRPKALQPPCNLLMQSEEVEDSGGARRSVIGS    77
hACS5     1 ----------------------MLFIF-NFLFSPLPTPALICILTFGAA--IFL-WLI-TRPQPVLP--LLDLNNQSVGIEGGARKGVSQK    62
hACS4     1 ------------------------------------------------MAKR-IKAKPTSDKPGSPYRSVTHFDSLAVIDIPGADTL    38
                                                          NH2 ←→ LR1
hACS1    78 SDEPL-VYFYDDVTTLYEGFQRGIQVSNNG-PCLG-SRKPDQP-YEWLSYKQVAELSECIGSALIQKGFKTAPDQFIGIFAQNRPEWVII   163
hACS2    78 GP-QLLTHYYDDARTMYQVFRRGLSISGNG-PCLG-FRKPKQP-YQWLSYQEVADRAEFLGSGLLQHNCKACTDQFIGVFAQNRPEWIIV   163
hACS5    63 NNDLTSCC-FSDAKTMYEVFQRGLAVSDNG-PCLG-YRKPNQP-YRWLSYKQVSDRAEYLGSCLLHKGYKSSPDQFVGIFAQNRPEWIIS   148
hACS4    39 DKLFDHAVSKFGKKDSLGTREI-LSEENEMQPNGKVFKKLILGNYKWMNYLEVNRRVNNFGSGLTALGLK--PKNTIAIFCETRAEWMIA   125

hACS1   164 EQGCFAYSMVIVPLYDTLGNEAITYIVNKAELSLVFVDKPEKAKLLLEGVENKLIPGLKIIVVMDAYGSELVERGQRCGVEVTSMKAMED   253
hACS2   164 ELACYTYSMVVVPLYDTLGPGAIRYIINTADISTVIVDKPQKAVLLLEHVERKETPGLKLIILMDPFEEALKERGQKCGVVIKSMQAVED   253
hACS5   149 ELACYTYSMVAVPLYDTLGPEAIVHIVNKADIAMVICDTPQKALVLIGNVEKGFTPSLKVIILMDPFDDDLKQRGEKSGIEILSLYDAEN   238
hACS4   126 AQTCFKYNFPLVTLYATLGKEAVVHGLNESEASYLITSVELLESKLKTALLD-ISCVKHIIYVDNKAINKAEYPEGFEIHSMQSVEELGS   214

hACS1   254 LGRANR-RKPKPPAPEDLAV-ICFTSGTTGNPKGAMVTHRNIVSDCSAFVKATEN-TVNP-CPDDTLISFLPLAHMFERVVECVML-CHG   338
hACS2   254 CGQENH-QAPVPPQPDDLSI-VCFTSGTTGNPKGAMLTHGNVVADFSGFLKVTES-QWAPTCADVHI-SYLPLAHMFERMVQSVVY-CHG   338
hACS5   239 LGKEHF-RKPVPPSPEDLSV-ICFTSGTTGDPKGAMITHQNIVSNAAAFLKCVDH-AYEPTPDDVAISY-LPLAHMFERIVQAVVYSC-G   323
hACS4   215 NPENLGIPPSRPT-PSDMAIVM-YTSGSTGRPKGVMMHHSNLIAGMTGQCERIPGLGPKD--TY--IGYL-PLAHVLELTAEISCFTY-G   296
                                                  LR1 ←→ Linker                    Linker ←→ LR2
hACS1   339 AKIGF-----FQGDIRLLMDDLKV----LQPTVFPVVPRLLNRMFDRIFGQANTTLKRWL--LDFASKRKEAELRSGIIRNNSLWDRLIF   417
hACS2   339 GRVGF-----FQGDIRLLSDDMKA----LCPTIFPVVPRLLNRMYDKIFSQANTPLKRWL--LEFAAKRKQAEVRSGIIRNDSIWDELFF   417
hACS5   324 ARVGF-----FQGDIRLLADDMKT----LKPTLFPAVPRLLNRIYDKVQNEAKTPLKKFL--LKLAVSSKFKELQKGIIRHDSFWDKLIF   402
hACS4   297 CRIGYSSPLTLSDQSSKIKKGSKGDCTVLKPTLMAAVPEIMDRIYKNVMSKVQEMNYIQKTLFKIGYDYKLEQIKKGYDAPLCNLLL--F   384

hACS1   418 HKVGSSLGGRVRLMVTGAAPVSATVLTFLRAALGCQVYEGYGQTECTAGCCLTMPGDWTAGHVGAPMPCNLIKLVDVEEMNYMAAEGE--   505
hACS2   418 NKIQASLGGCVRMIVTGAAPASPTVLGFLRAALGCQVYEGYGQTECTAGCTFTTPGDWTSGHVGAPLPCNHIKLVDVEELNYWACKGE--   505
hACS5   403 AKIQDSLGGRVRVIVTGAAPMSTSVMTFFRAAMGCQVYEAYGQTECTGGCTFTLPGDWTSGHVGVPLACNYVKLEDVADMNYFTVNNE--   490
hACS4   385 KKVKALLGGNVRMMLSGGGPLSPQTHRFMNVCFCCPIGQGYGLTESCGAGTVTEVTDYTTGRVGAPLICCEIKLKDWQEGGYTINDKPNP   474

hACS1   506 -GEVCVKGPNVFQGYLKDPAKTAEALDK--DG--WLHTGDIGKWLPNGTLKIIDRKKHIFKLAQGEYIAPEKIENIYMRSEPVAQVF-VH   589
hACS2   506 -GEICVRGPNVFKGYLKDPDRTKEALDS--DG--WLHTGDIGKWLPAGTLKIIDRKKHIFKLAQGEYVAPEKIENIYIRSQPVAQIY-VH   589
hACS5   491 -GEVCIKGTNVFKGYLKDPEKTQEALDS--DG--WLHTGDIGRWLPNGTLKIIDRKKNIFKLAQGEYIAPEKIENIYNRSQPVLQIF-VH   574
hACS4   475 RGEIVIGGQNISMGYFKNEEKTAEDYSVDENGQRWFCTGDIGEFHPDGCLQIIDRKKDLVKLQAGEYVSLGKVEAALKNC-PLIDNICAF   563
                                                                                  LR2 ←→ COOH
hACS1   590 GESLQAFLIAIVVPDVETLCSWAQKRGFEGSFEELCRNKDVKKAILEDMVRLGKDSGLKPFEQVKG-ITLHPEPFSIDNGLLTPTMKAKR   678
hACS2   590 GDSLKAFLVGIVVPDPEVMPSWAQKRGIEGTYADLCTNKDLKKAILEDMVRLGKESGLHSFEQVKA-IHIHSDMFSVQNGLLTPTLKAKR   678
hACS5   575 GESLRSSLVGVVVPDTDVLPSFAAKLGVKGSFEELCQNQVVREAILEDLQKIGKESGLKTFEQVKA-IFLHPEPFSIENGLLTPTLKAKR   663
hACS4   564 AKSDQSYVISFVVPNQKRLTLLAQQKGVEGTWVDICNNPAMEAEILKEIREAANAMKLERFEIPIKVR-LSPEEWTPETGLVTDAFKLKR   652

hACS1   679 PELRNYFRSQIDDLYS-TIKV                                                                      698
hACS2   679 PELREYFKKQIEELYSIPM--                                                                      697
hACS5   664 GELSKYFRTQIDSLYEHIQD-                                                                      683
hACS4   653 KELRNHYLKDIERMYGGK---                                                                      670
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**FIG. 1.** Organization of the human ACS4 gene. (A) Schematic diagram of overlapping λ and BAC clones encoding the human ACS4 gene. Exons are indicated by numbers and closed boxes. ACS4 protein is schematically represented at the bottom of the diagram. LR1 and LR2, luciferase-like regions 1 and 2, respectively. (B) Comparison of the exon/intron organization of the human ACS4 gene with those of human ACS1, ACS2, and ACS5 genes. The five domains in the ACS family are labeled as follows: N-terminal ($NH_2$), LR1, a linker connecting two luciferase-like regions (Linker), LR2, and C-terminal (COOH). Arrowheads indicate the positions at which introns interrupt exons.

**TABLE 1**

Exon/Intron Organization of the Human ACS4 Gene

| Exon No. | Exon size (bp) | Sequence at exon–intron junction | | Amino acid interrupted | Intron length (kb) |
|---|---|---|---|---|---|
| | | 5′ Splice donor | 3′ Splice acceptor | | |
| 1 | 145 | GCC CCT CCG gtgagtatccct | ctcttactgcagATT GAA ATC | | 37.0 |
| 2 | 53 | AGG ACA TTT gtaagtatttgt | accaatgccaagAAG AAA AAC | | 12.0 |
| | | F K K | L I L | | |
| 3 | 240 | TTT AAG AAG gtaaagtatttt | tcactttaacagTTA ATT CTT | Lys76 | 0.2 |
| | | F P L | V T | | |
| 4 | 178 | TTT CCT C gtaagtgccttg | tttattatgtag TT GTG ACT | Leu136 | 1.3 |
| | | K L K | T A L | | |
| 5 | 110 | AAA CTT AAG gtaaatatgaaa | tctgctcaacagACT GCA TTG | Lys172 | 0.1 |
| | | E N L | G I | | |
| 6 | 139 | GAA AAC T gtaagtaaagca | tcattcatttag TG GGC ATT | Leu219 | 2.6 |
| | | G L G | P K | | |
| 7 | 151 | GGA CTG GG gtaagataattc | attttgttttag A CCG AAG | Gly269 | 0.2 |
| | | S D Q | S S K | | |
| 8 | 124 | TCT GAC CAG gtgacaaacttt | tctttaatatagTCC AGC AAA | Gln310 | 3.5 |
| | | A V P | E I M | | |
| 9 | 72 | GCT GTT CCG gtgagtacagaa | tttaactttcagGAA ATC ATG | Pro334 | 5.2 |
| | | C N L | L L | | |
| 10 | 140 | TGC AAT CT gtaagtacattt | ccctttattaag G TTA CTG | Leu381 | 0.8 |
| | | T E V | T D | | |
| 11 | 173 | ACT GAA G gtaagcattatt | tttccctcacag TA ACT GAC | Val439 | 2.6 |
| | | Q E G | G Y | | |
| 12 | 75 | CAA GAA G gtaagaatttta | tatgatttccag GC GGT TAT | Gly464 | 2.0 |
| | | I I D | R K | | |
| 13 | 192 | ATT ATA G gtcagaatgttt | atgacttaacag AT CGT AAG | Asp528 | 1.6 |
| | | A K S | D Q | | |
| 14 | 115 | GCC AAA AG gtaactttccaa | ctgtttttttag T GAT CAG | Ser566 | 2.0 |
| | | N A M | K L | | |
| 15 | 158 | AAT GCC A gtaagtaagaaa | ttctaatttcag TG AAA TTG | Met619 | 15.2 |
| 16 | 2854 | | | | |

*Note.* Exon sequences and intron sequences are in uppercase and lowercase letters, respectively. Introns are positioned by applying the GT/AT rule (Breathnach *et al.*, 1978).

PE Biosystems). To analyze RNA in cancer cell lines, commercially available Northern blots (Clontech) were hybridized with the entire coding region of the human ACS4 cDNA labeled with [$\alpha$-$^{32}$P]dCTP by random priming methods (10).

*Genomic clones encoding the human ACS4 gene.* Recombinant bacteriophage clones were isolated by plaque hybridization from a genomic DNA library of normal human peripheral leukocytes in EMBL3 vectors using the entire region of the human ACS4 cDNA labeled with [$\alpha$-$^{32}$P]dCTP. Bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) clones containing the human ACS4 genes were obtained by PCR-based screening of a BAC library (Research Genetics, Huntsville, AL) and human genomic Mega YAC libraries A, B, and C from the Centre d'Etude du Polymorphisme Humain (CEPH) (11), respectively. A set of human ACS4 specific primers, 5′-AAAAAGGAAGCAAAGGAGAC-3′ [sequence in the (+) strand of exon9] and 5′-CATGTATCAGCAAACTCAAA-3′ [sequence in the (−) strand of intron 9], were used under the conditions recommended for Ex Taq (Takara Shuzo Corp.). DNA fragments carrying exons were identified by restriction mapping and Southern blotting. After subcloning into pBluescript vectors, the sequences of exons, exon/intron boundaries, and the 5′-flanking region were determined. Intron sizes of the human ACS4 gene were determined by Southern blotting, restriction mapping, and PCR analysis.

*Reporter plasmid constructs.* To test for promoter activity, various lengths of the 5′-flanking region of exon 1 were fused to the firefly luciferase gene present in pGV-B2 (Toyo Ink Mfg. Co., Ltd., Tokyo), which contains no eukaryotic promoter or enhancer elements. An *Eco*RI/*Nhe*I fragment containing 5′-upstream region of exon 1 was subcloned into the *Eco*RI/*Xba*I sites of pBlueScriptII KS(+), and sequenced completely. A DNA fragment, containing −891 to +61, was generated by digestion with *Not*I, blunt-ending by Klenow fragment, and digestion with *Kpn*I. This fragment was inserted into the *Kpn*I/*Sma*I sites of pGV-B2 to create a reporter plasmid, phACS4(−891). A series of deletion reporter plasmids was generated by digestion of phACS4(−891) with *Kpn*I and *Eco*RI, digestion with exonuclease III, blunt-ending with Klenow fragment, and ligation with T4 DNA ligase. The sequences of these serial deletion reporter plasmids were confirmed by nucleotide sequencing.

*DNA transfection.* HeLa S3 cells were cultured in EMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 $\mu$g/ml streptomycin. $3 \times 10^5$ cells were transfected with 0.8 $\mu$g of test plasmid and 0.2 $\mu$g of the sea pansy (*Renilla reniformis*) luciferase expression plasmid pRL-TK (Toyo Ink Mfg. Co., Ltd.), using FuGENE6 (Roche Diagnostics Inc.) reagent according to the manufacturer's recommendations. Cells were harvested 48 h after transfection for the measurement of luciferase activities. In the transfection experiment, parallel plates of HeLa S3 cells were transfected with pGV-B2 and pGV-P2 (Toyo Ink Mfg. Co., Ltd.), which serve as negative and positive controls, respectively. The plasmid pGV-B2 lacks a eukaryotic promoter, and apparently no luciferase activity was detected in the HeLa S3 cells transfected with pGV-B2. The

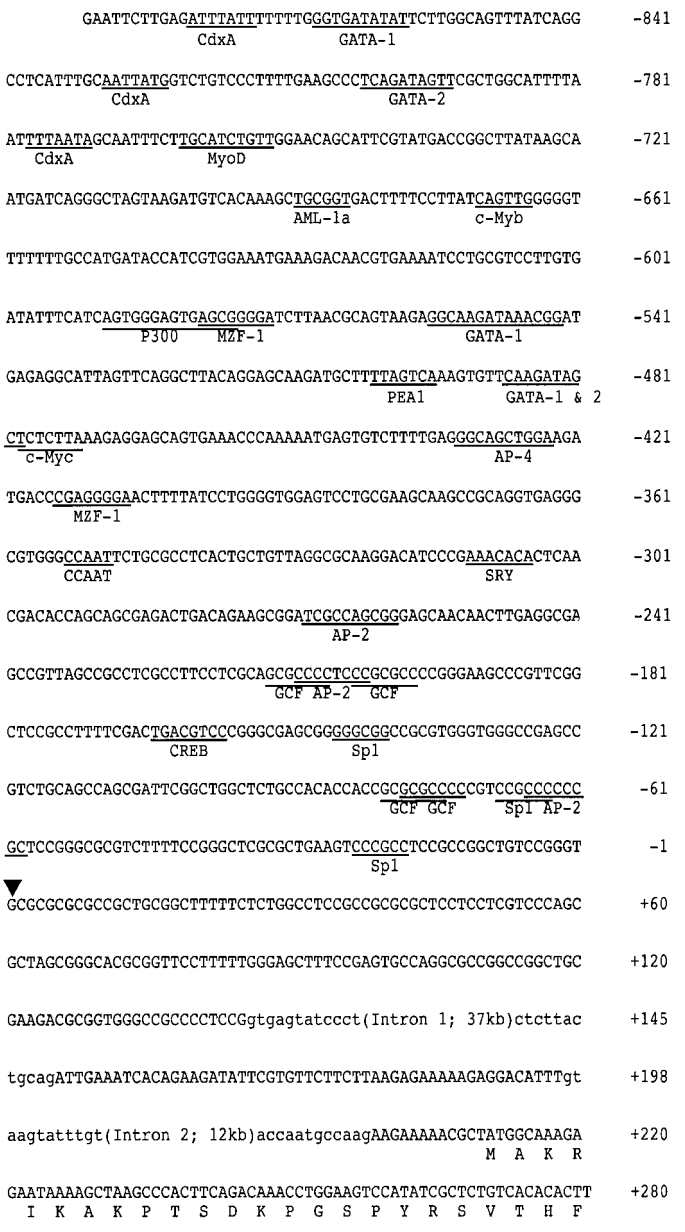


**FIG. 2.** Nucleotide sequence of the 5′-flanking region of the human ACS4 gene. Nucleotide 1 corresponds to the G, 210 nucleotides upstream of the initiator methionine codon. The residues preceding it are indicated in negative numbers. An arrowhead above the sequence indicates the transcription start site as determined by primer extension. Potential binding sites for transcription factors (>90 cutoff score) are underlined.

plasmid pGV-P2 contains the SV40 early promoter driving the expression of the luciferase gene.

*Firefly luciferase and sea pansy luciferase assays.* Transfected cells were washed three times with phosphate-buffered saline. Cell lysis, firefly luciferase assay, and sea pansy luciferase assay were performed using PicaGene Dual SeaPansy Luminescence Kit (Toyo Ink Mfg. Co., Ltd.) and LUMINOUS CT-9000D luminometer (DIAIATRON). The protein content of the cell extract was measured by the method of Lowry *et al.* (12).

## RESULTS AND DISCUSSION

*Isolation and characterization of genomic clones encoding the human ACS4 gene.* As an initial step for the characterization of the human ACS4 gene, we isolated a human ACS4 cDNA (designated phACS4–5B; GenBank Accession No. AB061714) from a HepG2 cDNA library using rat ACS4 cDNA as a probe. The coding sequence of phACS4–5B was completely identical to that reported by Piccini *et al.* (7) and Cao *et al.* (13), and contained 210 bp 5′-untranslated region (UTR) from the AUG translation initiator codon. Using this plasmid as a probe, we screened a human genomic library constructed in the EMBL3 vector. Screening of $3 \times 10^5$ clones using phACS4–5B as a probe, three positive clones (Fig. 1A; λhACS4–10, λhACS4–11, and λhACS4–15) were obtained. Analysis of these clones by Southern blotting and nucleotide sequencing revealed that these clones did not contain exon 1 (encoding a portion of the 5′-UTR) of the gene. To obtain clones containing this region, we screened human BAC libraries using ACS4-specific PCR and obtained five BAC clones (302O15, 419B16, 435L15, 462M10, and 495L12); the presence of the ACS4 sequence in each clone was confirmed by Southern blotting. One representative clone (495L12), containing exon 1 and the 5′-flanking region of the gene, was further characterized. Characterization of these genomic clones revealed that the gene spans approximately 90 kb encoding 16 exons. Sequences at all the exon/intron junctions conformed to the GT/AG rule (Table 1). The relevant sequence information is available under GenBank Accession Nos. AB061696 to AB061711.

*Genomic organization of the human ACS4 gene.* Figure 1A summarizes the genomic organization of the human ACS4 gene. Exons 1 and 2 encode the 5′-UTR, and exon 3 contains the translation initiator codon AUG. Exon 16 contains the translation termination codon and the 3′-UTR. Functionally important luciferase-like regions LR1 and LR2 are encoded by exons 4–10 and 11–16, respectively. Figure 1B compares the amino acid positions that are interrupted with introns of the human ACS4 gene with those of the human ACS1, ACS2, and ACS5 (13) genes. The exon/intron organizations of human ACS1 and ACS2 genes were determined using the human genome draft sequence entries of the GenBank databases (GenBank Accession Nos. AC084871 and AC079257 for the human ACS1 gene and AC034228 for the human ACS2 gene). Although the positions interrupted in the ACS4 gene differ slightly from those of the human ACS1, ACS2, and ACS5 genes, in which almost the same positions are interrupted, the exon/intron organization of the ACS4 gene closely resembles those of the human ACS1, ACS2, and ACS5 genes. These data suggest that all the ACS genes may have evolved from a common ancestral gene.

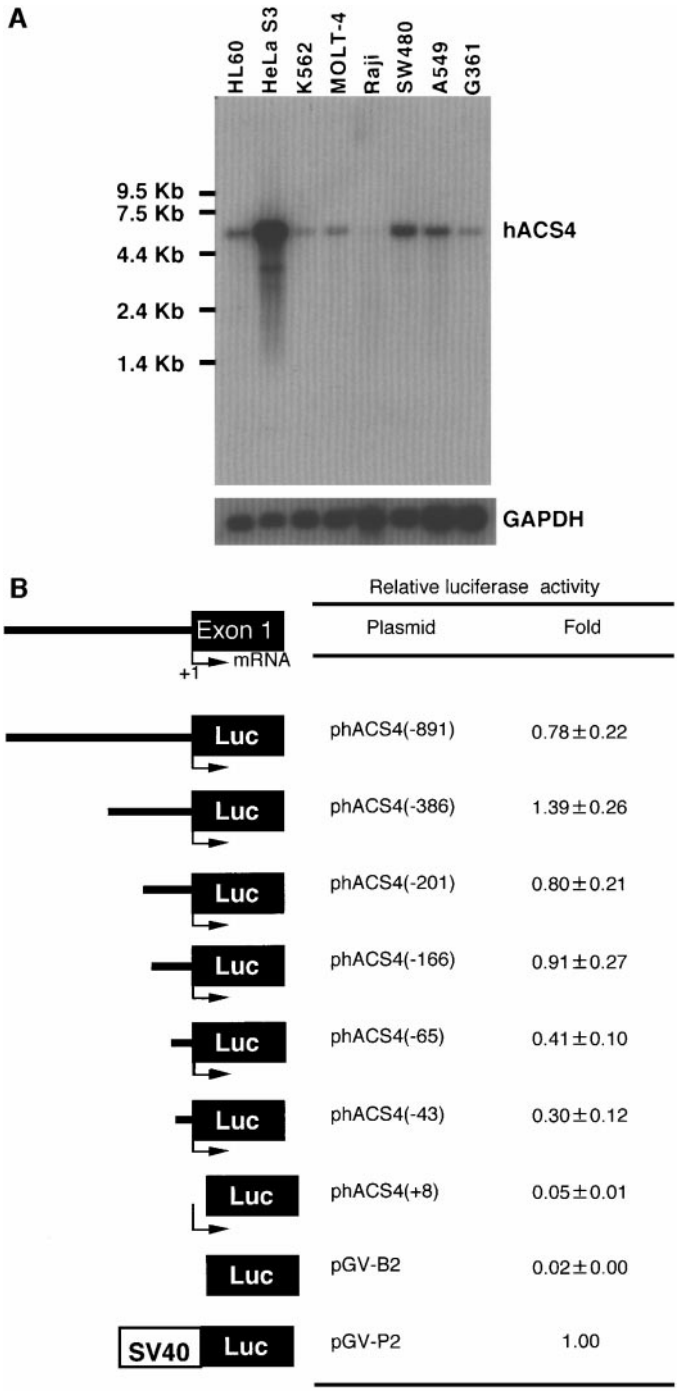


**FIG. 3.** Analysis of the promoter activity. (A) Expression of ACS4 transcripts in human cancer cell lines. Approximately 2 $\mu$g of poly(A) RNA from the indicated tissue and cell line was probed with $^{32}$P-labeled human ACS4 cDNA. The filter was exposed to Kodak XAR-5 film with an intensifying screen at −80°C for 18 h. Control hybridization with human GAPDH probe is shown at the foot of the figure. (B) Functional promoter activity of the 5′-flanking region of the human ACS4 gene fused to the firefly luciferase reporter gene. Varying lengths of the 5′-flanking region of the gene were fused to the luciferase gene in pGV-B2 (see Experimental Procedures). Each chimeric gene was co-transfected with the sea pansy expression plasmid (pRL-TK) into HeLa S3 cells, and assayed for firefly luciferase and sea pansy luciferase activities as described under Experimental Procedures. Firefly luciferase activity in each individual experiment was corrected for variation in transfection efficiency by normalizing the value to the sea pansy luciferase activity in the same extract. The normalized activity of each promoter was then expressed relative to that of pGV-P2, with pGV-P2 assigned a relative activity of 1.0. The data represent the mean of triple transfection experiments for each plasmid.

*Characteristics of the 5′-flanking region of the human ACS4 gene.* In describing the 5′-flanking region of the human ACS4 gene, we used a numbering scheme in which the transcription start site of the gene is designated +1. Based on the 5′-end of phACS4–5B and on the primer extension analysis (data not shown), the transcription site was assigned to the G, 210 nucleotides upstream of the initiator methionine codon.

Figure 2 shows the nucleotide sequence of the 5′-flanking region of the human ACS4 gene. Computer-assisted identification of putative promoter/enhancer elements (set at a cutoff score of >90%) was performed using MOTIF (http://motif.genome.ad.jp/), and TFSEARCH (http://pdap1.trc.rwcp.or.jp/research/db/TFSEARCHJ.html). Neither a typical TATA box (15) sequence, nor its homologue was found in the 5′-flanking region, but a CCAAT box (16) is present at −354. Potential sites for Sp1 (17) are present at −146, −69 and −24. The 5′-flanking region also contains DNA motifs for AP-2 (18) at −269, −210, and −66, AP-4 (19) at −312, and CREB (20) at −165. The presence of CREB site near the transcription start site is noteworthy, since the levels of ACS4 mRNA and protein in mouse Y1 adrenocortical tumor cells were increase by dibutyryl cAMP (db-cAMP) and forskolin (6). Other potential sites for CdxA (21), MZF-1 (22), p300 (23), SRY (24), MyoD (25), AML-1a (26), c-myb (27, 28), c-myc (29), PEA1 (30), GC box (31), and GATAs (32, 33) are also present in the 5′-flanking region.

*Functional analysis of the 5′-flanking region.* Prior to testing the functional promoter activity of the 5′-flanking region, we analyzed the ACS4 transcripts in cell lines. Northern blotting of human cancer cell lines with the human ACS4 probe revealed that the expression of ACS4 is the highest in HeLa S3 cells (Fig. 3A).

To determine the region required for the expression in HeLa S3 cells, the genomic DNA fragment containing the 5′-flanking region (−891 to +61) was fused to the luciferase gene in pGV-B2 to create phACS4(−891). Following transfection into HeLa S3 cells, this chimeric gene produced significant luciferase activity relative to the vector, indicating that this fragment contains a functional promoter (Fig. 3B). In order to determine a minimal promoter region, a series of 5′-deletions was introduced into this promoter region. Deletion of the sequence from −891 to −386 increased promoter activity approximately twofold, suggesting that this region contains a negative promoter element. A further deletion to −201 resulted in an approxi-

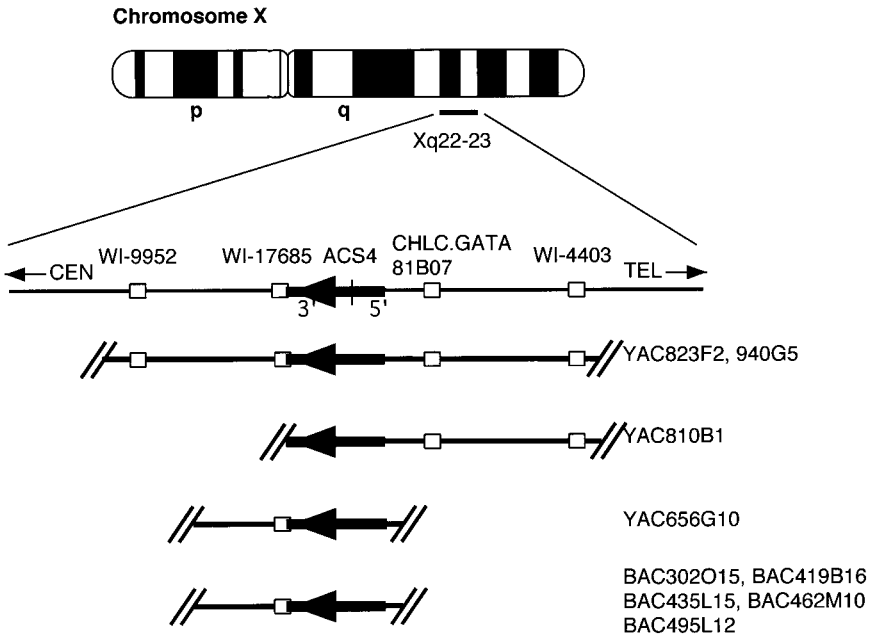


**FIG. 4.** Localization of the human ACS4 gene on the YAC contig map. Human chromosome region Xq22–23 is represented schematically at the top of the diagram. Five YAC and five BAC clones used for the analysis of the human ACS4 gene are represented. The arrows indicate the human ACS4 gene and its transcriptional direction. CEN, centromere; TEL, telomere. The locations of the STS markers are from the RHdb databases (http://www.ebi.ac.uk/RHdb/).

mately 40% loss of promoter activity, suggesting that a positive promoter element is contained between −386 and −201. A further deletion to −43 resulted in complete loss of promoter activity. These data indicate that a minimal promoter includes the 159 bp (−201 to −43) relative to +1. This region contains potential binding sites for Sp1, AP-2, GCF, and CREB (Fig. 2). These potential sites may be required for the basal transcription and/or regulation of the human ACS4 gene. Further studies are required to elucidate the regulation of the ACS4 gene.

*Fine mapping of the human ACS4 gene.* Our initial mapping of the human ACS4 gene by fluorescence *in situ* hybridization (FISH) revealed the localization of the gene on Xq22–23 (data not shown), consistent with the previous studies (7, 13). To further define the location of the gene on chromosome X, human YAC libraries were screened by ACS4-specific PCR. Four YAC clones (656G10, 810B1, 823F2, and 940G5) were positive for this screening, and the existence of the ACS4 gene in each clone was confirmed by Southern blotting using hACS4 cDNA as a probe. Two of the four clones (810B1 and 940G5) were mapped between DXS1210 and DXS1001 according to the STS content map (Data Release 12, July 1997; WICGR ChrX) of Human Genomic Mapping Project, Whitehead Institute/MIT Center for Genome Research (http://waldo.wi.mit.edu/ftp/distribution/human_STS_releases/july97/pictures/). PCR analysis with STS marker specific primers revealed that YAC clones, 810B1, 823F2, and 940G5 contained two STS markers, WI-4403 and CHLC.GATA81B07 (Fig. 4). An STS marker, WI-17685 (the STS-Based Map of the Human Genome, http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map) neighboring CHLC.GATA81B07, was present in clones 656G10, 823F2, and 940G5. We also analyzed five BAC clones encoding the human ACS4 gene (302O15, 419B16, 435L15, 462M10 and 495L12), and found the presence of WI-17685 in these clones. Furthermore, sequence analysis of a BAC clone (495L12) revealed the presence of WI-17685, approximately 20 kb downstream of the polyadenylation site of the human ACS4 gene (sequence not shown). BLAST searches of the databases, using the human ACS4 sequences, identified three overlapping human genome draft sequence entries (GenBank Accession Nos. AL138968, AL118496, and AL590647). The two complete sequence entries, AL138968, and AL118496, contain exons 1–9, and exons 9–16 and two STS markers (WI-17685 and WI-9952) respectively. An incomplete sequence entry, AL590647, contains exons 2–9, 5′-flanking region, and an STS marker, CHLC.GATA81B07. Taken together, the human ACS4 gene was mapped between the STS markers, WI-17685 and CHLC.GATA81B07 on Xq22–23 region. Our mapping data will be helpful to understand the functional consequences of the ACS4 gene deletion in human X-linked diseases including Alport syndrome.

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