Characterization of the gene for the mouse prostaglandin E receptor subtype EP_2: tissue-specific initiation of transcription in the macrophage and the uterus

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Genomic DNA clones for the mouse prostaglandin (PG) E receptor subtype EP_2 were isolated and characterized. The mouse EP_2 gene is composed of 2 exons and 1 intron, and spans 16 kb. The intron which is approx. 12 kb in length is located at the end of the sixth transmembrane domain, as with other prostanoid receptor genes. Based on this structure, transcripts were analysed in endotoxin-treated macrophages and pseudopregnant uteri, in which abundant expression of EP_2 mRNA was observed. Sequence analysis of cDNA clones from these origins and Northern hybridization of these RNAs revealed that the uterine EP_2 mRNA (U-type) has a longer 5′-untranslated region than the macrophage EP_2 transcript (M-type). The major transcription initiation sites for M-type and U-type EP_2 are located 124 and 769 bp upstream of the translation start site, respectively. The M-type was expressed in various tissues, whereas the U-type was found only in the uterus. The 2 kb segment containing the immediate 5′-flanking and 5′-noncoding regions contain three consensus sequences for the NF-IL6 binding site, one consensus sequence for the NF-κB binding site, four AP-2 consensus sequences, one AP-4 consensus sequence, one potential cAMP response element, and one potential progesterone response element. These results suggest that EP_2 gene expression in the macrophage and uterus is under the control of distinct mechanisms involving alternative promoters.

INTRODUCTION

Prostaglandin (PG) E_2 produces a broad range of biological actions in various tissues through binding to specific receptors on plasma membranes [1,2]. The PGE receptor is pharmacologically divided into four subtypes, EP_1, EP_2, EP_3, and EP_4, which differ in their signal transduction mechanisms; these receptors are coupled to Ca^{2+} mobilization (EP_1), or the stimulation (EP_2 and EP_4) or inhibition (EP_3) of adenylate cyclase [3–5]. We previously isolated cDNAs encoding these four subtypes of the PGE receptor [6–10], and characterized their structural, binding, and signalling properties and tissue distribution. Although both EP_2 and EP_4 are coupled to the stimulation of adenylate cyclase, expression of EP_2 mRNA is much lower than that of EP_4 in almost all tissues. However, abundant EP_2 mRNA expression was observed in the macrophage and uterus. EP_2 mRNA is induced in the luminal epithelium of the mouse uterus on day 5 of pseudopregnancy, suggesting that the EP_2 gene is hormonally regulated in the uterus [11]. The dominant EP_2 transcript detected in the uterus was 2.8 kb in length. On the other hand, EP_2 mRNA in J774.1 cells, a macrophage-like cell line, is highly induced by stimulation with lipopolysaccharide (LPS) (unpublished data). In this cell line, the major EP_2 transcript detected was 2.2 kb in length, as was observed in tissues other than the uterus. These observations lead to the hypothesis that expression of EP_2 is induced by hormonal or pathophysiological stimuli, and that distinct activation systems of EP_2 gene expression exist in these two types of stimuli. As an initial step to clarify the mechanisms of EP_2 induction, we isolated and characterized genomic DNA clones encoding EP_2. We further found differences in transcription initiation of the EP_2 gene between the macrophage and uterus.

EXPERIMENTAL

Isolation of genomic clones

A mouse genomic library (approx. 5.0×10^5 recombinants from the 129SVJ λFIXII library, Stratagene) was screened by hybridization with a 466 bp fragment of the mouse EP_2 cDNA spanning the putative third to sixth transmembrane domains. Hybridization was carried out under the conditions described previously [12]. Ten positive clones were isolated and subjected to sequence and restriction analyses. These clones contained the nucleotide sequence upstream of the sixth transmembrane domain of the mouse EP_2 but not the seventh transmembrane and 3′-untranslated regions. Therefore, the library was then screened with a 543 bp *Hin*cII fragment covering the putative seventh transmembrane and C-terminal regions. Five other genomic clones were isolated and subjected to sequence and restriction analyses. Nucleotide sequencing was carried out on both strands by the dideoxy-chain termination method. Intron size was determined

Abbreviations used: PG, prostaglandin; LPS, lipopolysaccharide; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction; CRE; cAMP response element; PRE, progesterone response element; LE cell, luminal epithelial cell.

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The nucleotide sequences reported in this paper will appear in the DDBJ/EMBL/GenBank DNA databases under the accession numbers AB007695 and AB007696.

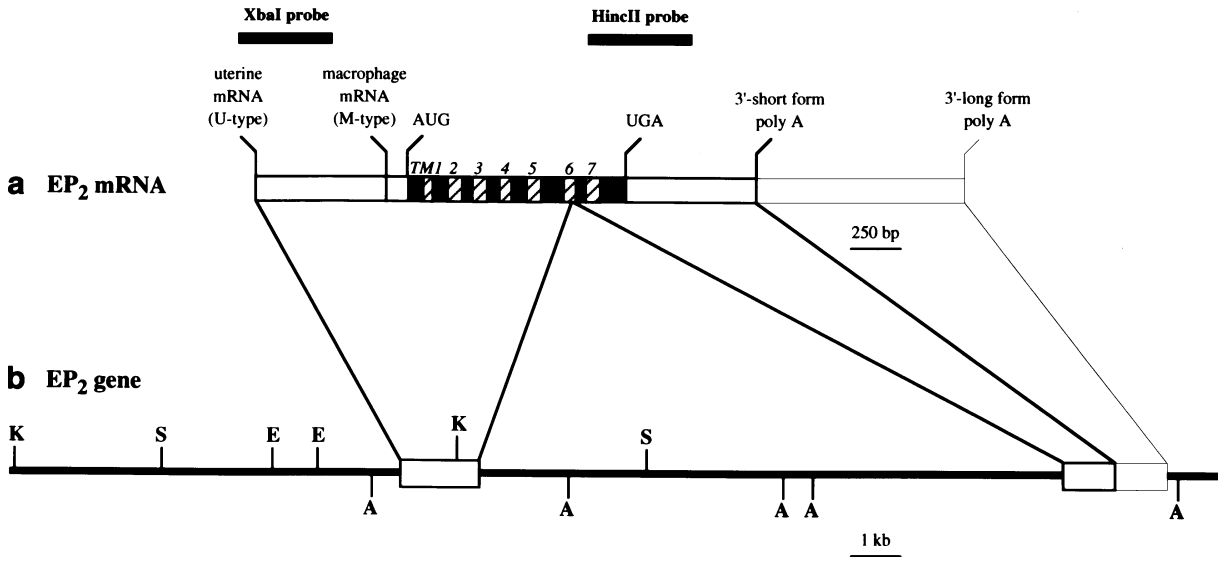

Figure 1 Schematic diagram of the mouse EP_2 mRNA and gene

(**a**) Structure of the mouse EP_2 mRNA. The noncoding regions are represented by open boxes, the putative transmembrane regions are indicated by hatched boxes and numbered, and the other coding regions are shown by closed boxes. The translation initiation site, AUG, the translation termination site, UGA, and two polyadenylation sites are indicated. Positions of probes used in the Northern blot analysis are depicted by bold bars and labelled. (**b**) A diagram of the mouse EP_2 gene. The restriction sites are indicated as follows: *Aat*I (A), *Eco*RI (E), *Kpn*I (K), *Sma*I (S). Exons are represented by open boxes.

by PCR using mouse liver genomic DNA as the template and the LA PCR Kit Ver. 2 (Takara Shuzo, Kyoto, Japan). Oligonucleotides (35-mer) corresponding to the exon–intron junctions of the EP_2 gene were used as PCR primers, and amplification was performed under these conditions in the following order; (1) 94 °C for 1 min, (2) 98 °C for 10 s and 68 °C for 15 min for 14 cycles, (3) 98 °C for 10 s and 68 °C for 15 min, with an addition of 15 s/cycle at 68 °C for 16 cycles, (4) 72 °C for 10 min. The amplified DNA fragment was subjected to restriction analysis.

Isolation of cDNA clones from a mouse uterus cDNA library

A mouse uterus cDNA library was prepared by the oligo(dT)-priming method. Approx. 4.0×10^5 recombinant clones from this library were screened by hybridization with the insert DNA of ML202 [10]. Thirteen positive clones were isolated and subjected to sequence and restriction analyses.

Northern blot analysis

J774.1 macrophage-like cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). The cells were treated with 100 ng/ml LPS (B *E. coli* 055:B5, Difco Laboratories, Detroit, MI, U.S.A.) for 3 h. Pseudopregnancy was achieved in virgin mice essentially as described previously [11]. Total RNA was isolated from LPS-treated J774.1 cells and pseudopregnant day-5 uteri by the acid guanidinium thiocyanate–phenol–chloroform method [13]. The RNAs (10 μg) were separated by electrophoresis on a 1.5% agarose gel, and transferred onto a nylon membrane (Biodyne, Pall Biosupport Division, East Hills, NY, U.S.A.). Hybridization probes used were as follows; a *Hin*cII fragment (543 bp) covering the seventh transmembrane and C-terminal regions, a *Xba*I fragment (469 bp) corresponding to the 5′-noncoding region of uterine EP_2 cDNA clones, and an *Eco*RI/*Sph*I fragment (377 bp) of the 3′-terminus of an EP_2 cDNA clone with a long 3′-untranslated region (ML204). Hybridization was carried out under the conditions described previously [6].

Determination of the transcription start sites

The two antisense oligonucleotides used in primer extension analysis were the EP2NR primer corresponding to nucleotides +27 to −3 of ML202 [10] and the EP2UR primer corresponding to 610–644 bp upstream of the ATG start codon. The primers were labelled with [γ-^{32}P]ATP (DuPont–NEN) by T4 polynucleotide kinase. Poly(A)$^+$ RNAs were purified from LPS-treated J774.1 cells and pseudopregnant day-5 uteri using Oligotex dT30 'Super' (Takara Shuzo, Kyoto, Japan). The ^{32}P-labelled EP2NR primer was annealed overnight to 5 μg of poly(A)$^+$ RNAs in hybridization buffer (40 mM Pipes, pH 6.4, 1 mM EDTA, 0.4 M NaCl, 80% formamide) at 30 °C. The ^{32}P-labelled EP2UR primer was annealed to 10 μg of poly(A)$^+$ RNAs. The annealed RNA/primer mixture was extended by Superscript II reverse transcriptase (Life Technologies) at 42 °C for 1 h. The extension products were electrophoresed on a 6% polyacrylamide gel containing 7 M urea. Resultant fragment sizes were determined by sequencing ladders generated by the same primers.

The 5′-rapid amplification of cDNA ends (RACE) experiment was performed essentially as described previously [14]. Poly(A)$^+$ RNA (1 μg) from LPS-treated J774.1 cells was reverse transcribed with the EP2NR primer, and the resultant first-strand cDNA was polyadenylated by terminal deoxynucleotidyl transferase. The cDNA was amplified with an oligo(dT) primer (25-mer) and the EP2NR3 primer complementary to nucleotides 96–125 of ML202. The resultant products were then amplified using another primer EP2NR4, complementary to nucleotides 71–95 of ML202. The amplified products were separated on a 2% agarose gel, and two major products, approx. 350 and 270 bp in length, were subcloned into a plasmid vector for sequencing.

Detection of EP_2 mRNA variants by the reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNAs from various tissues were transcribed with M-MLV reverse transcriptase (Life Technologies) using hexanucleotides as primers, and the resultant cDNAs were used for PCR. For the detection of 5′-end variants, amplification was performed with the EP2EXR primer complementary to the 35 nucleotides at the 5′-terminus of exon 2, in combination with either the MG2721 primer corresponding to nucleotides −99 to −72 or the MG2723 primer corresponding to nucleotides −456 to −429. A uterine EP_2 cDNA clone (MU14) was used as a positive control of the amplification. For the amplification of 3′-end variants, PCR was performed with the EP2EXF primer corresponding to the 35 nucleotides at the 3′-terminus of exon 1, in combination with either the EP2MJR primer complementary to nucleotides 840–864 of exon 2 or the ML204R4 primer complementary to nucleotides 955–984 of exon 2. ML204 was used as a positive control of the amplification. Amplified products were separated by electrophoresis on 1% agarose gels.

RESULTS

Structure of the mouse EP_2 gene

Structural analysis of isolated clones revealed that the mouse EP_2

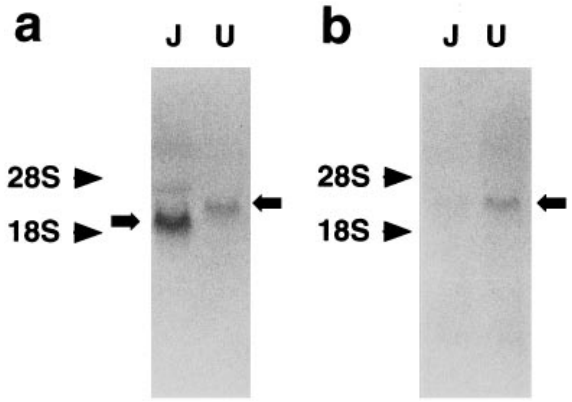

Figure 3 Northern blot analysis of RNAs isolated from LPS-treated J774.1 cells and pseudopregnant day-5 mouse uteri

LPS treatment of J774.1 cells and achievement of pseudopregnancy in virgin mice were performed as described in Experimental. Total RNA (10 μg) was applied to each lane. (**a**) EP_2 mRNAs were detected by a 543 bp *Hin*cII fragment covering the seventh transmembrane and C-terminal regions. A major band was detected at 2.2 and 2.8 kb in J774.1 cells and uteri, respectively. (**b**) A 469 bp *Xba*I fragment from the 5′-noncoding region of uterine EP_2 cDNA was used as a probe. Only the 2.8 kb transcript was detected. J, LPS-treated J774.1 cells; U, pseudopregnant day-5 uteri.

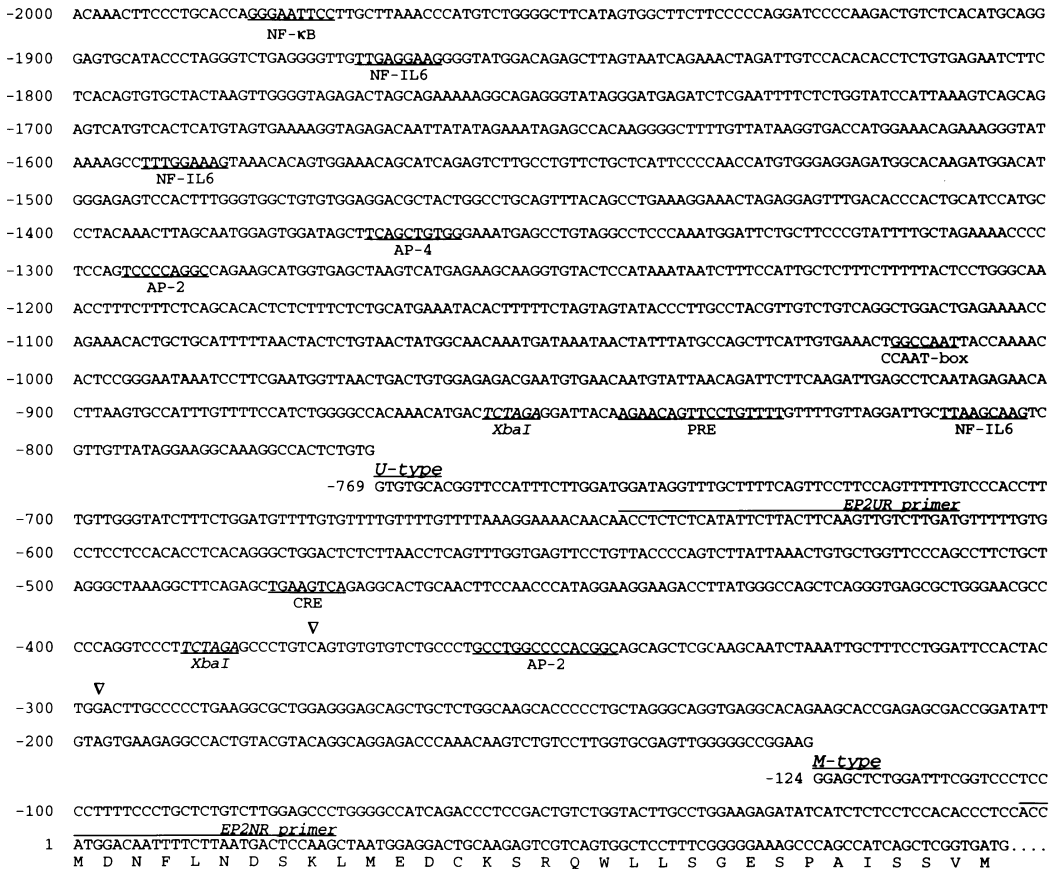

Figure 2 Nucleotide sequence of the 5′-flanking and 5′-noncoding regions of the mouse EP_2 gene

Numbers on the left are relative to the adenosine in the ATG start codon. Representative cDNA clones ML202 and MU14 start at positions −145 and −743, respectively. The major transcription initiation sites for macrophage (M-type) and uterine (U-type) transcripts are indicated. Two other potential transcription start sites for macrophage transcripts are shown by open triangles. Potential binding sites for the indicated transcription factors are underlined. The two *Xba*I restriction sites are italicized and underlined. Locations of the primers used in primer extension analysis are shown by lines above the sequence.

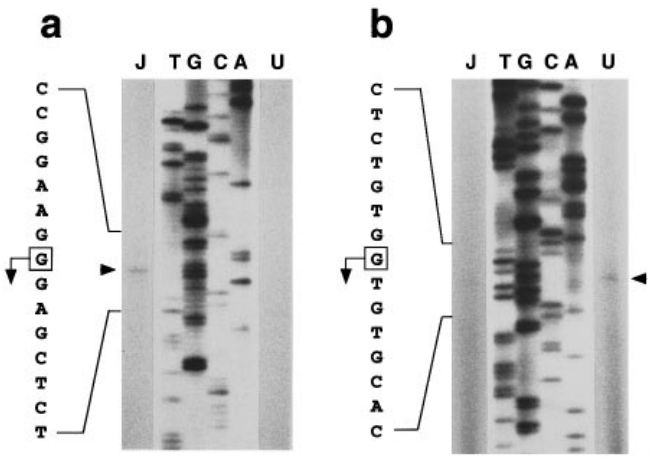

Figure 4 Determination of the transcription start sites of the mouse EP_2 gene by primer extension analysis

^{32}P-labelled antisense oligonucleotides were annealed to poly(A)$^+$ RNAs as described in the Experimental section (**a** and **b**) Extension by EP2NR and EP2UR primers, respectively. Arrowheads indicate the extended products. J, LPS-treated J774.1 cells; U, pseudopregnant day-5 uteri.

gene spans 16 kb in length and contains 2 exons (Figure 1). Exon 1 is composed of the 5′-untranslated region and the coding region from ATG to the putative sixth transmembrane domain of the receptor. Exon 2 encodes the residual part of the coding region and the 3′-untranslated region. The sequences of the exons were in complete agreement with that of the cDNA. The exon–intron boundaries conform perfectly with the GT/AG rule (data not shown) [15,16]. In Southern blot analysis of mouse genomic DNA, digestion by *Bam*HI, *Nco*I, *Nde*I, or *Sca*I yielded a single hybridization band, suggesting that the EP_2 is specified by a single gene (data not shown). Using the genomic DNA structure as a basis, we further characterized the mRNA species from the macrophage and uterus.

Characterization of cDNAs from a mouse uterus library

In a previous study we reported that the 2.2 kb species of EP_2 mRNA was distributed in various tissues, whereas the 2.8 kb transcript was abundantly expressed only in the uterus, and hence appeared to be uterus-specific [10]. To obtain structural information on the uterus-derived EP_2 transcript, we isolated EP_2 cDNA clones from a mouse uterus cDNA library. Thirteen positive clones were isolated, and nine clones contained the full-length of the coding region. Restriction analysis of these clones revealed that the sequence of the coding region was the same as that of ML202, a representative EP_2 cDNA clone derived from the lung [10]. However, 5 out of 9 uterine clones contained an additional upstream genomic sequence (640–545 bp in length) at their 5′-ends compared with the lung cDNAs (Figures 1 and 2). Owing to these findings, we further examined whether the additional sequence observed only in the uterine EP_2 cDNA corresponds to the extra sequence in the uterine-specific 2.8 kb transcript.

Northern hybridization with region-specific probes

The preliminary investigation showed that the expression of the 2.2 kb EP_2 transcript was higher in LPS-treated J774.1 cells, a macrophage-like cell line, than in the lung. To examine whether

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   1 ATCTTTGCCTACATGGATGAAACCTCTTCCCTAAAGGAAAAGTGGGACCTCCGAGCTCTTCGGTTTTTATCAGTTAACTCTATAATTGAC
 282 I F A Y M D E T S S L K E K W D L R A L R F L S V N S I I D
     VI                                                    VII

  91 CCTTGGGTCTTTGCCATACTTAGGCCACCGGTCCTGAGGTTAATGCGCTCAGTCCTCTGTTGTCGGACTTCACTGAGAACACAAGAAGCT
 312 P W V F A I L R P P V L R L M R S V L C C R T S L R T Q E A

 181 CAGCAAACATCCTGTTCTACCCAGTCCAGTGCCAGTAAACAGACTGACCTTTGTGGACAGTTGTGAGGATGCGCTTCACGAGGGAACCTC
 342 Q Q T S C S T Q S S A S K Q T D L C G Q L

 271 TGAAGAAGTCTTTAAATGGCCTCATTGGAGAAGTGTAAGGGGCTGGAATATAAACAGAATAACCTTGCCCTGAGAAGCAGATGAAACAGA
 361 CTTTATGAGGTTTGGGCTGCAGATGTGATGACAGAGCCCTTTGTGGAAAGTGTCAGAGGATATAAAGTTCACATTATGTGACCTTTGAAG
 451 GACAATCTGCTGCGTCGAAGACCCAGTTGATTTAAGCTTTCCTGTTGAGTGACAAAGCATGTGGTTTTGTAATTTGTTTTAAAATGCTAC
 541 ATGGTTACTGTTTTAATTTTGATGTCACTGCTGTTATAAATGCAGTCAGACCAAATGAAATTCATTCTCTAGGAAGTCAACTCATGCAAG
 631 CGATGAAGCAACCAGAGCAGACAAGTTGCTGTCTCGTGATTGCTTAATGGACTCTTTATTTGGACCATGAAGTTGAATGCCATTGGCCCC
 721 TTTTACTGTGCGTGTTCATGTGGGAGTCCTCTCTGGTAACGAAAGGATGCACTGATTTGGTTAATGCTAGTTAATCCTTTAGGAGCTCTG
 811 TCATGTTGTCAGATTATTTATTCTGTCTTGTCTATTGTGATAAGACTACTGAAGAAAACTTCAGAAATTTGTGTCTCACAAAGGAATAAT
 901 TAAAAAAATCAATTTT  (3'-short form poly A site)

                  AATTAGTGTTCTTCAGTTTTTTATAGAGGAAGAGTTTTCACAGTTGTATCTGAGATCAGATTTTAGGATCAAAC
 991 TCTGAAAATTCAAGTCTATTTTTCAGGTTCTCTAGAATCCTTCTAGTGTTGGTACTAGACATAACTAACATACACATACACTACAAGATG
1081 TTTGATTGTGCCACGGCAAACGCTTTTGTCTGTATTTTTATTGAGGGAACATATTTTGATTCATACAACAAAAGAAATCATGTAACTGTG
1171 AGTCATATCTTATATTTCCAAACGTTTGGTACGGTATAAACTCAGCATTAAAGTATTCCAGTGAGCGTGTACATTTAGTCAGTTATAGTG
1261 TAAACACATCTGAAATGTTACAAGCTAAATTATAAAACAAAAAAGTAGAAGTCTTCAATTAATTCACTCCCCAAGGACAGTTTGCAGTTA
1351 ATGTAGACATGGCATCTTTTAGGTAAAATAAAAGTTTTTAAAAAATTCAATAATATTTTGGCAAGTTTTTAAAATGTACAGTTGTTATAA
1441 ATTAAAATTTTAAGATATCCAGTGGAGTTCTTGATATTGAAAGAGAGTTTTGCTATCAGTTTTCACTCTTCTGACATGGTCATTAGAATA
1531 ATATAACCATCGTATTTGAGGGCAGATAGAATTGTAGGATGGCTGTTAACTTTTTAAATTTAGTTGTTTCTCATGCTTCATGTTTACCCA
1621 GAGCTGTTTTTTATAGTTAGTTATCATTTAGAAAGACTCTGTGTAACTAAATATCTCTGATACAAATTATGGGAGTCAAAGCAGCTCTTA
1711 CTTTTACAAGAATATATAAATAAGTGGTTCGTGTTTGTATAAATTTAACAGAGCAGACAAAGTTTAATTTAACTAAAATCTAGGCAGGTA
1801 TAAATAAATGCCACCTCTTCACCCGGTGTTGCATGCCAAGAAATTACATTCAGGGGGAGAATGAGGCCCGTGGTATGACACTGAAGCAGA
1891 AATTTCTGGTTACTTTGCACCTGGAATTTTGATTCCTTTTATCAGATGTCGTTGTAAAAATCTCAGAATAAAAAAAAATTAACTAC
                                                                    (3'-long form poly A site)
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Figure 5 Nucleotide sequence of exon 2 of the mouse EP_2 gene

The deduced amino acid sequence is shown beneath the nucleotide sequence using the single-letter code. The positions of the putative transmembrane segments, VI and VII, are underlined. AATAAA polyadenylation signals are double underlined. ATTTA mRNA destabilizing motifs are italicized and underlined. The previously reported cDNA clone ML202 stopped at nucleotide 724 and was polyadenylated.

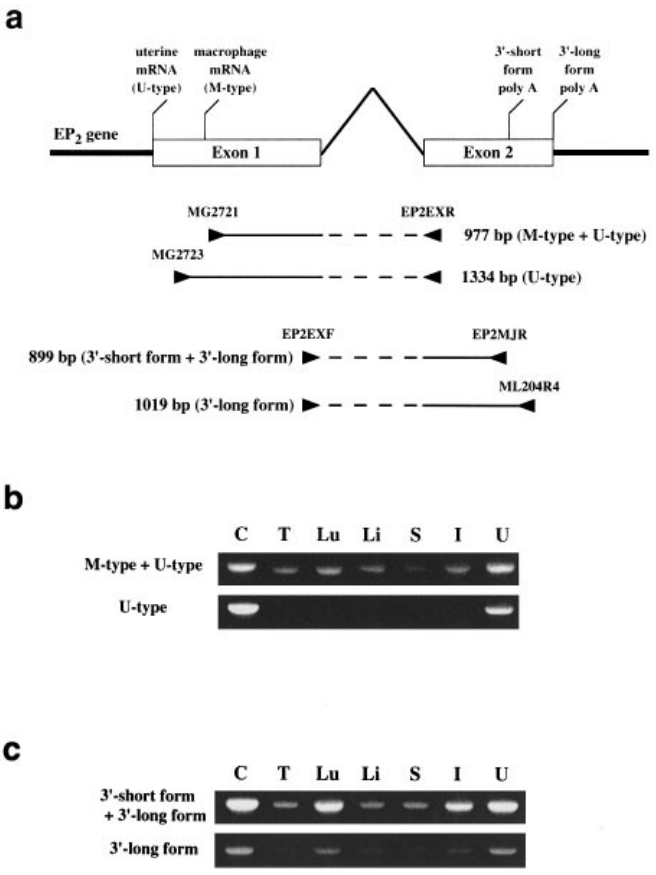

Figure 6 Tissue specificity of EP_2 mRNA variants

RT-PCR was performed as described in the Experimental section (**a**) Diagram of the locations of the PCR primers and amplified products. The MG2721 primer in combination with the EP2EXR primer amplifies both the M-type and U-type EP_2 transcripts, and a 977 bp fragment is detected. On the other hand, the MG2723 primer amplifies only the U-type mRNA and a 1334 bp fragment is detected. As for the 3′-end variants, the EP2MJR primer in combination with the EP2EXF primer amplifies both the 3′-short form and the 3′-long form, and an 899 bp fragment is detected. On the other hand, the ML204R4 primer amplifies only the 3′-long form, and a 1019 bp fragment is detected. (**b**) Distribution of the two types of 5′-end variants. A uterine EP_2 cDNA clone was used as a control of the amplification. The M-type transcript is expressed in various tissues, whereas the expression of U-type mRNA is restricted to the uterus. (**c**) Distribution of two forms of 3′-end variants. ML204 was used as a control of the amplification. The long form is expressed in all tissues examined. C, control DNA; T, thymus; Lu, lung; Li, liver; S, spleen; I, ileum; U, pseudopregnant day-5 uterus.

the uterus-specific 2.8 kb transcript is derived from the difference in the length of the 5′-noncoding sequence, we performed Northern blot analysis on the J774.1 cells and uteri using a probe specific for the additional sequence. We first used a *Hin*cII fragment covering the seventh transmembrane and C-terminal regions of EP_2 as a probe. As shown in Figure 3a, a major band was observed at 2.2 and 2.8 kb in LPS-treated J774.1 cells and pseudopregnant day-5 uteri, respectively. In J774.1 cells, a minor band was also observed at 4.0 kb. On the other hand, when a *Xba*I fragment specific for uterine EP_2 cDNA was used as a probe, the transcripts from the J774.1 cells were not detected (Figure 3b). These results showed that only the uterine EP_2 transcript has an additional 5′-untranslated region, suggesting that the transcription of the uterine EP_2 mRNA starts approx. 600 bp upstream of that in the lung.

Determination of the transcription start site

In order to confirm uterus-specific transcription initiation, we next investigated the transcription initiation sites for the macrophage (M-type) and uterus (U-type) EP_2 transcripts. Primer extension analysis was first performed with two antisense oligonucleotides EP2NR (corresponding to position +27 to −3) and EP2UR (position −610 to −644). When EP2NR was used as a primer, an extended product was detected in RNA from J774.1 cells, which started at the guanine nucleotide at position −124 (Figure 4a). The product was not detected in RNA from the uterus. As shown in Figure 4b, when EP2UR was used as a primer, an extended product was detected in RNA from the uterus, which started at the guanine nucleotide at position −769. This product was barely detected in RNA from J774.1 cells. Thus, the major transcription initiation sites from M-type and U-type EP_2 were identified as positions −124 and −769, respectively. Two additional minor transcription initiation sites for the M-type were further identified by the 5′-RACE experiment (Figure 2). From these results we conclude that the transcription start site of uterine EP_2 mRNA is approx. 600 bp upstream of that in the other tissues.

Diversity of the EP_2 transcripts in their 3′-noncoding region

Heterogeneity was also found in the length of the 3′-noncoding sequence in EP_2 cDNA (Figures 1 and 5). When cloning the EP_2 cDNA from a mouse lung library, isolated clones were subdivided into three groups, represented by ML202, ML203, and ML204, according to their 3′-terminal lengths. Eleven out of 12 clones from the uterus library had the same 3′-tail as ML203, suggesting that this type of 3′-terminal structure is the dominant form in the uterus (Figure 5, 3′-short form). The other clone, MU6, had the same 3′-tail structure as ML204 (Figure 5, 3′-long form). This mRNA species contained not only three polyadenylation signals, AATAAA [17], but also five mRNA destabilizing motifs, ATTTA [18]. To examine whether the 4.0 kb minor band detected in J774.1 cells corresponds to the 3′-long form, we performed Northern hybridization with a probe specific for the 3′-long form. However, significant levels of transcripts were not detected with this probe (data not shown), suggesting that the 3′-long form was expressed at a very low level, or was susceptible to degradation. The 4.0 kb minor band detected in J774.1 cells might be an immature form of the 2.2 kb mRNA.

Tissue distribution analysis of EP_2 mRNA variants

Tissue specificity of the expression of EP_2 mRNA with a long 5′- or 3′-untranslated region was examined by RT-PCR (Figure 6). As for the 5′-end variants, when the MG2721 primer, which amplifies both the M-type and U-type EP_2, was used, a 977 bp fragment was detected in all tissues examined. In contrast, when amplified with the MG2723 primer specific for the U-type, a 1334 bp fragment was detected only in pseudopregnant day-5 uteri (Figure 6b). These results suggest that the M-type was expressed in various tissues, whereas the U-type was expressed exclusively in the uterus. As for the 3′-end variants, the long form was expressed in all tissues examined (Figure 6c), suggesting that tissue specificity does not exist in the expression of the EP_2 mRNA with the long 3′-untranslated region.

Analysis of the promoter sequence

The 2 kb nucleotide sequence containing the immediate 5′-flanking and 5′-noncoding regions of the mouse EP_2 gene was examined (Figure 2). No consensus TATA-box sequence was

found in the presumed promoter region of either the M-type or U-type EP_2. Although one consensus TATA-box sequence is present at position −1603, it seems too distant to be involved in transcription. A CCAAT-box is present 247 bp upstream of the major transcription start site for the U-type. Consensus motifs relevant to LPS stimulation, such as NF-IL6 and NF-κB binding sites, are found in the 5′-flanking region. Consensus sequences for the NF-IL6 binding site are present at positions −811, −1593 and −1871. One consensus sequence for the NF-κB binding site is found at position −1982. In addition, in the promoter region for the M-type, one putative cAMP response element (CRE) and a cluster of three AP-2 consensus sequences were found at positions −480 and −352, −353, and −359, respectively. On the contrary, no apparent consensus sequence related to hormone-dependent expression in the uterus, such as an oestrogen response element (ERE) or a progesterone response element (PRE), was found in the 5′-flanking region. However, one potential PRE was found at position −844, 75 bp upstream of the major transcription start site for the U-type, In addition, one AP-2 consensus sequence and one AP-4 consensus sequence are present at positions −1295 and −1370, respectively.

DISCUSSION

In this study, we characterized the structure of the mouse EP_2 gene and the diversity of its transcripts in various tissues and cells. The mouse EP_2 gene is composed of 2 exons. Exon 1 contains the 5′-untranslated region, the translation initiation codon, and most of the EP_2 receptor coding region. Intron 1, which is approximately 12 kb in length, divides the coding region at the putative sixth transmembrane domain. The location of this intron in the sixth transmembrane domain is completely conserved in the genes of eight types and subtypes of prostanoid receptors [14,19–24]. On the contrary, the EP_2 gene does not have an intron in the 5′-noncoding region, which was found to be present in five of the prostanoid receptors. Appearance of an intron in this region may depend on the type of expression control in each gene. Among prostanoid receptors, the human TP receptor gene and the bovine FP receptor gene have been reported to generate two mRNA species by alternative promoter usage [19,25]. However, these two mRNA species appeared to be co-expressed in the same tissue. In this study, we identified diversity of EP_2 mRNA in both the 5′- and 3′-ends. RT-PCR and Northern analyses revealed that the 3′-end variants are co-expressed in various tissues and that the short form is dominant. On the other hand, expression of the 5′-end variants is tissue-specific; 5′-long (U-type) mRNA is expressed only in the uterus and 5′-short (M-type) mRNA is expressed in the other tissues and the macrophage. Thus, the EP_2 gene performs tissue-specific transcriptional initiation. Significance of the long 5′-noncoding sequence of the U-type is yet unknown, but it might be involved in the alteration of the stability or translation efficiency of the transcript. Indeed, the 5′-noncoding region of the U-type contains four AUG codons. Presence of these start codons upstream of the main open reading frame is known to inhibit cap-dependent translation [26]. Therefore, the translation efficiency of the U-type might be lower than that of the M-type. Strict control of translation, in addition to transcriptional regulation, might be necessary for precise expression of EP_2 in the uterus.

Previously, we found that in the synchronized mouse uterus, EP_2 mRNA is induced in the luminal epithelial (LE) cells only during the peri-implantation period [11]. This observation suggests the involvement of PGE_2 and the EP_2 receptor in blastocyst implantation. Further analyses using ovariectomized mice revealed that the expression of EP_2 mRNA was induced in uterine LE cells by exogenously-added progesterone (unpublished data), suggesting that uterine expression of the EP_2 gene is under the control of ovarian progesterone. In this study, we concluded that uterine transcription of the EP_2 gene starts 645 bp upstream of its initiation site in the macrophage cell line. This type of transcription initiation was observed only in the uterus, suggesting that a uterus-specific promoter is involved in regulation of EP_2 gene expression. One potential PRE was found in the 5′-flanking sequence, 75 bp upstream of the uterine transcription start site. Since the activity of uterine cells is highly regulated by ovarian steroids during the peri-implantation period, the PRE in the EP_2 gene may be involved in activation of the closer transcription start site in uterine LE cells in response to progesterone.

Expression of the 2.2 kb species of the EP_2 transcript was observed in various tissues other than the uterus. In addition, this EP_2 transcript was induced by LPS stimulation in J774.1 cells. PGE_2 is known to inhibit LPS-induced cytokine production in the macrophage via cAMP accumulation [27]. LPS-induced EP_2 might be involved in such inhibitory actions of PGE_2 in the activated macrophage. Indeed, in the 2 kb immediate 5′-flanking region of the EP_2 gene, three consensus sequences for the NF-IL6 binding site and one consensus sequence for the NF-κB binding site were found (Figure 2). It is possible that the 2.2 kb transcript is induced by these inflammatory transcription factors [28,29]. It is interesting in this respect that the expression levels of the 2.2 kb transcript were extremely low in the healthy mouse tissues except uterus.

The most remarkable finding in this study is that uterine transcription initiation of the EP_2 gene is different from that in other tissues. This suggests that distinct promoters are involved in EP_2 gene expression in the uterus and other tissues. Such distinct and separate mechanisms between hormonal and other controls of transcription can be observed in the expression of the mouse FP receptor gene; the 7.3 kb fragment of the 5′-flanking region contains enough promoter sequence for its expression in the stomach and kidney, but not in the corpora lutea [14]. In the case of the EP_2 gene, however, it remains unknown at present whether the uterine promoter and the promoter for other tissues work independently from each other. It is also unclear whether some of the putative promoter regions in both systems overlap and share some basic transcription factors. Thus, further systematic studies on EP_2 gene expression in the uterus and other tissues are essential.

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