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Identification of the promoter region of human interleukin 1 type I receptor gene: Multiple initiation sites, high G+C content, and constitutive expression

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ABSTRACT **To better understand the role of interleukin 1 (IL-1) and its receptor in disease, we have isolated a genomic clone of the human IL-1 type I receptor and have identified the promoter region. There are multiple transcriptional initiation sites as demonstrated by primer extension. DNA sequence analysis shows that the promoter region contains neither a TATA nor a CAAT box; however, the 5′ upstream regulatory elements contain two AP-1-like binding sites. The internal regulatory sequences found immediately downstream to the 5′ transcriptional start site contain four Sp1 binding domains and have a high G+C content of 75%. This portion of the 5′ untranslated region of the mRNA can form stable secondary structure as predicted by computer modeling. Base pairs −4 to +10 share striking resemblance to an initiator sequence that directs basal expression of certain TATA-less genes—e.g., terminal deoxynucleotidyltransferase in lymphocytes. The IL-1 receptor promoter directs basal expression of chloramphenicol acetyltransferase in transiently transfected cells. Overall, the promoter of the IL-1 type I receptor gene resembles that of constitutively expressed genes that have housekeeping- and/or growth-related functions. The constitutive nature of the promoter may account for this gene being expressed at low levels in diverse cell types. Our finding sheds more understanding into the mechanisms governing the regulation of the IL-1 receptor in health and disease.**

The diverse biological effects of interleukin 1 (IL-1) are determined by the interaction of IL-1 and its receptors. Although two IL-1 receptors have been cloned (1–3), the regulation of receptor synthesis remains unknown. The type I receptor (IL-1RI) has a 213-amino acid cytoplasmic domain (2) and is fully functional for signal transduction (4), whereas the type II receptor (IL-1RII) has only a short cytoplasmic domain of 29 amino acids (3). It is not known whether the type II receptor can transduce a signal or is a nonfunctional receptor.

IL-1RI has wide tissue distribution and may play a dominant role in terms of biological function. The level of expression of IL-1RI varies with different cell types. IL-1RI is overproduced in certain T-cell lines (5); however, the significance and mechanism of this overproduction are not clear. In other cell types, the expression of the type I receptor is low (for review, see ref. 6). Regulation of IL-1RI likely plays an important role in controlling the effects of IL-1; increasing the IL-1RI number by 1000-fold in Chinese hamster ovary cells leads to an increase of 1000-fold in sensitivity to IL-1 (4). It is now known that a number of factors influence the level of receptors. Among the cytokines, IL-2 and IL-4 upregulate IL-1RI in T-cell lines (7, 8), and platelet-derived growth factor and interferonγ increase the receptor level in fibroblasts and keratinocytes, respectively (9, 10). To investigate the regulation of expression of the IL-1RI gene at the molecular level, we cloned, identified, and characterized the 5′ flanking region of this gene.†

MATERIALS AND METHODS

Screening of Human Genomic Library. A human placental genomic library was purchased from Clontech. This library was prepared by partial *Sau*3A digestion and cloned into the *Bam*HI site of EMBL-3 vector. Recombinant phage (10^6) were screened from the library through hybridization with a human IL-1RI cDNA probe (from position 1 to 959, a 5′ *Xba* I fragment) (11). An oligonucleotide (GAAGCTGGACACCCCTTGGT) corresponding to the 5′ untranslated region (UTR) of the cDNA was synthesized and end-labeled as a probe. Southern hybridization of genomic clones with this probe verified that they contained this sequence. Genomic clone 29 contained sequences homologous to the human IL-1RI cDNA and was the longest clone obtained. It was selected and further subcloned into pGEM-3Z plasmid (Promega).

DNA Sequence Analysis. Different subclones of clone 29 were subjected to restriction analysis and Southern blot hybridization using IL-1RI cDNA as a probe. Hybridization-positive fragments of appropriate size were ligated into M13mp18 and M13mp19 bacteriophage and used for dideoxynucleotide DNA sequencing. Dideoxynucleotide DNA sequencing kits were purchased from United States Biochemical and sequencing was performed as described by the manufacturer. DNA homology searches of GenBank version 60 were performed by using the University of Wisconsin Genetics Computer Group (version 5) on a Vax computer. The mRNA secondary structure was also generated by using the FOLD program that determines a secondary structure of minimum free energy for an RNA sequence on the basis of published values of stacking and loop-destabilizing energies (12).

Primer Extension. Primer extension was performed by using two oligonucleotide primers (30-mers) complementary to two sequences in the 5′ UTR of the human IL-1RI mRNA; these sequences are ACGCACCCTCTGAAGATGGTGACTCCCTCC and TCGCCCTCTGAGCTGAGCCGGGTTCCGCCC (oligo 1 and oligo 2, respectively). [γ-^{32}P]ATP end-labeled oligonucleotide primers (3×10^5 cpm) were annealed to total RNA (40 μg) and extended with reverse transcriptase (Promega) according to a standard protocol (13). The reaction samples were resolved under denaturing conditions on a 6% polyacrylamide sequencing gel alongside a set of dideoxy-

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Abbreviations: CAT, chloramphenicol acetyltransferase; UTR, untranslated region; IL-1, interleukin 1; IL-1RI, IL-1 type I receptor; SRE, serum response core element.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L09701).

nucleotide sequencing reactions to allow single base resolution of the extension products.

Chloramphenicol Acetyltransferase (CAT) Assay. Genomic fragments were inserted into plasmid pKK232-8, which contains a polylinker sequence upstream of the CAT gene (Pharmacia). For promoter CAT construction, a 976-bp *Bam*HI/*Eco*RI fragment was blunt ended with T4 polymerase on the *Eco*RI end and then the *Bam*HI/blunt fragment was isolated and subcloned into the *Sma* I-*Bam*HI site of pKK232-8 (named pKK976). The construct was transfected into the human monocytic cell line THP-1 (ATCC no. 202-TIB). Electroporations were carried out with a Gene-Pulser (Bio-Rad) with 10^7 cells and 15 μg of plasmid DNA (purified twice in CsCl/EtdBr gradients) at 200 V and 960 μF. Cells were incubated in RPMI 1640 medium containing 10% fetal calf serum for 48 h and then lysed by three freeze–thaw cycles, each consisting of a −70°C incubation (5 min) followed by 37°C for 5 min. The cell debris was removed by centrifugation at 13000 × *g* for 15 min, and the supernatant was assayed for CAT activity. Fifty microliters of cell lysate was added to 2 μl of [^{14}C]chloramphenicol (58 mCi/mmol; 1 Ci = 37 GBq; NEN) and 20 μl of 4 mM acetyl coenzyme A (Pharmacia). The reaction mixture was incubated for 1–2 h at 37°C and then extracted with 1 ml of ethyl acetate. The ethyl acetate was dried *in vacuo* and resuspended in 20 μl of fresh ethyl acetate, and spotted onto a thin-layer chromatography plate. Chromatography was carried out with chloroform/methanol (95:5). The plate was then exposed to x-ray film.

RESULTS

Restriction Map and Exon–Intron Organization of 5′-Specific Human IL-1RI Genomic Clone. Three overlapping but nonidentical genomic clones from a human placental library were obtained by using the human IL-1RI cDNA 5′ restriction fragment (*Xba* I digest) (11). One of the genomic clones (clone 29) was ≈14 kb and was the longest clone isolated; therefore, it was selected for further analysis. Based on the published cDNA sequence (2, 11), a synthetic oligonucleotide (GAAGCTGGACCCCTTGGT) corresponding to the 5′ UTR of the receptor was synthesized and end labeled as a probe. Southern hybridization confirmed that genomic clone 29 contains this 5′ sequence (data not shown). Clone 29 was then subcloned for restriction enzyme analysis and sequencing. Sequence analysis of two restriction fragments, a 1-kb *Eco*RI/*Pst* I and a 2.2-kb *Eco*RI/*Eco*RI digest revealed that one exon is located in each of these fragments. The 5′ UTR of the receptor is composed of two exons and an intron in the gene (Fig. 1*A*). Exon 1 was a 514-bp G+C-rich sequence separated by ≈10 kb of intron 1 followed by 77 bp of exon 2 that is most 5′ to the translational start site. Exon 2 had relatively conserved splicing signals on both 5′ and 3′ ends.

Primer Extension. To identify the transcription initiation site(s) of the IL-1RI gene, primer-extension analysis was performed with total mRNA from the human hepatoma cell line (HepG2) (14), human foreskin fibroblasts, and the human monocyte line THP-1 (15). Each of these cell lines expresses low levels of the type I receptor mRNA determined by Northern blot analysis (data not shown). When oligo 1 (see Fig. 1*B*) was end labeled with [^{32}P]ATP and extended with avian myeloblastosis virus reverse transcriptase, multiple bands were generated on a denatured sequencing gel (Fig. 2). The most abundant extension products were 518, 386, 282, and 204 bases 5′ to the primer (Fig. 2, oligo 1). These results suggest that transcriptional initiation of the IL-1RI gene may be heterogeneous, occurring 5′ upstream of exon 2. To accurately determine and verify the 5′ transcriptional start site, oligo 2, corresponding to the G+C-rich region of exon 1, was synthesized and end labeled (see Fig. 4). The extended

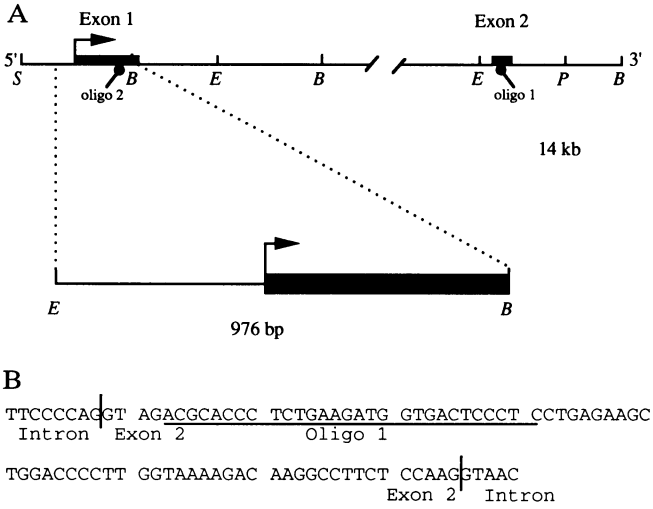

FIG. 1. (*A*) Restriction map of human IL-1RI genomic clone from λ phage clone 29. Solid boxes, exons 1 and 2; arrows, identified (solid) or possible (dotted) transcription start sites. A subcloned 976-bp *Eco*RI/*Bam*HI fragment is shown below the λ phage clone. (*B*) Complete sequence of exon 2 (77 bp). Vertical bars indicate exon–intron junctions. Oligo 1 used in primer extension is underlined. S, *Sac* I; E, *Eco*RI; B, *Bam*HI; P, *Pst* I.

products showed a single band (Fig. 2, oligo 2) 445 bases 5′ upstream of the primer. This band corresponded to band 1 from the previous extension reaction.

Predicted Secondary Structure For the 5′ UTR of the IL-1RI Gene. Unlike an earlier report (2) on the 5′ UTR sequence of IL-1RI from a T-cell library, the 5′ UTR sequence of the HepG2 cell (9) contains 225 additional bases upstream of the translational initiation codon as determined by cDNA sequence. These additional bases have an overall G+C content

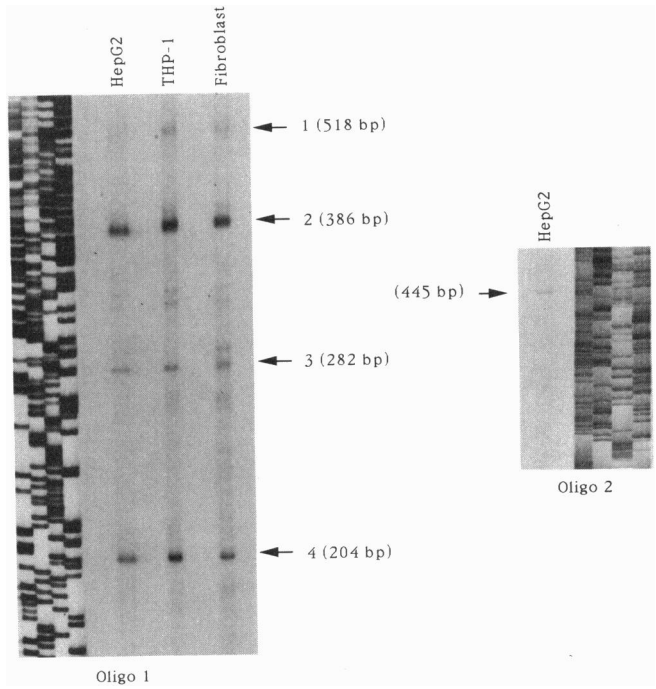

FIG. 2. Primer-extension study of human IL-1RI mRNA. Oligo 1 (see Fig. 1) was used to prime human HepG2, THP-1, and fibroblast total mRNA (40 μg). Reaction samples were resolved juxtaposed to a sequencing reaction. Arrows, major extension products (band 1, 518 bp; band 2, 386 bp; band 3, 282 bp; band 4, 204 bp). Oligo 2 (see Fig. 4) was also used to prime HepG2 cells and a single 445-bp extension product was detected (arrow).

of 80%. Using primer-extension analysis, our genomic clone contains 289 bp upstream of the above 225 bp for a total of 514 bp (75% G+C-rich). Computer analysis of these 514 bases of the IL-1RI mRNA was performed to predict an overall secondary structure of minimum free energy (Fig. 3). The calculated free energy at 37°C for the predicted structure is $\Delta G = -255.4$ kcal/mol (1 cal = 4.184 J). Particularly striking are several long repeats made up entirely of G or C residues that formed perfect complementary matches. This analysis suggests that multiple stable stem–loop structures can be potentially formed in the IL-1RI 5′ UTR. A 5′ UTR of >100 bases and with a high G+C content is relatively rare among mammalian mRNA but has been identified in a number of mRNAs encoding growth factors, membrane receptors, GTP-binding proteins, and several protooncogenes (16).

Sequence Analysis of the Promoter Region of the IL-1RI Gene. The nucleotide sequences upstream of the IL-1RI mRNA start sites contain neither a TATA nor a CAAT box (Fig. 4). Most eukaryotic genes contain one or both of these two elements approximately 30 and 80 bp upstream of the mRNA initiation site, respectively (17). Since the TATA element serves to localize the accuracy of initiation of transcription by the RNA polymerase II (18), it is not surprising that the IL-1RI gene has numerous transcriptional initiation sites. Interestingly, the sequences surrounding the transcriptional start site between nucleotides −4 to +10 share striking resemblance to the recently described initiator sequences that direct specific transcription initiation without a TATA sequence (19) (Fig. 5).

Further sequence analysis between nucleotides −489 and −1 that contain the putative promoter or upstream regulatory element revealed two AP-1-like sites at positions −242 (reversed) and −326 (Fig. 4). These two sequences bind AP-1 protein (20) [AP-1 consensus sequence TGA(G/C)T(C/A)A (21)]. A serum response core element (SRE) was also found at position −57 [SRE consensus sequence, CCATGG (22)]. Interestingly, within the putative promoter region of the IL-1RI gene, there are two A+T-rich regions of 15 and 16 bases (Fig. 4, underlined). In addition, the region immediately 3′ to the initiation site (also called internal regulatory sequences) contains four conserved Sp1 or Sp1-like binding domains at positions +8, +79, +107, and +215 [Sp1 consensus sequence, GGGCGG (21)]. These four putative Sp1 binding sites of the receptor promoter are within 250 bases 3′ of the most approximate initiation site (23, 24).

Constitutive Expression of the IL-1RI Gene Promoter. The ability of the putative promoter sequences described above and the most 5′ UTR sequences to support transcription was tested with the bacterial CAT structural gene in a transient expression assay. The *Eco*RI/*Bam*HI (976 bp; Fig. 1*A*) fragment from clone 29 was subcloned into pKK232-8 upstream of the CAT gene. The *Bam*HI site is located within the first exon, thus retaining the transcriptional start site but eliminating a portion of the cDNA sequence and the exon–intron junction (see Fig. 1*A*). This fragment of DNA was able to orchestrate the expression of the CAT reporter gene in THP-1 cells at a basal level (Fig. 6).

DISCUSSION

We have identified the human IL-1RI gene promoter in cloned genomic DNA. The IL-1RI gene does not contain a typical TATA or CAAT box, as do IL-1α (25), IL-1β (26), IL-1Ra (27), and most mammalian genes. The initiation of IL-1R mRNA transcription occurs at multiple sites. The 5′ flanking region, so-called upstream regulatory sequences,

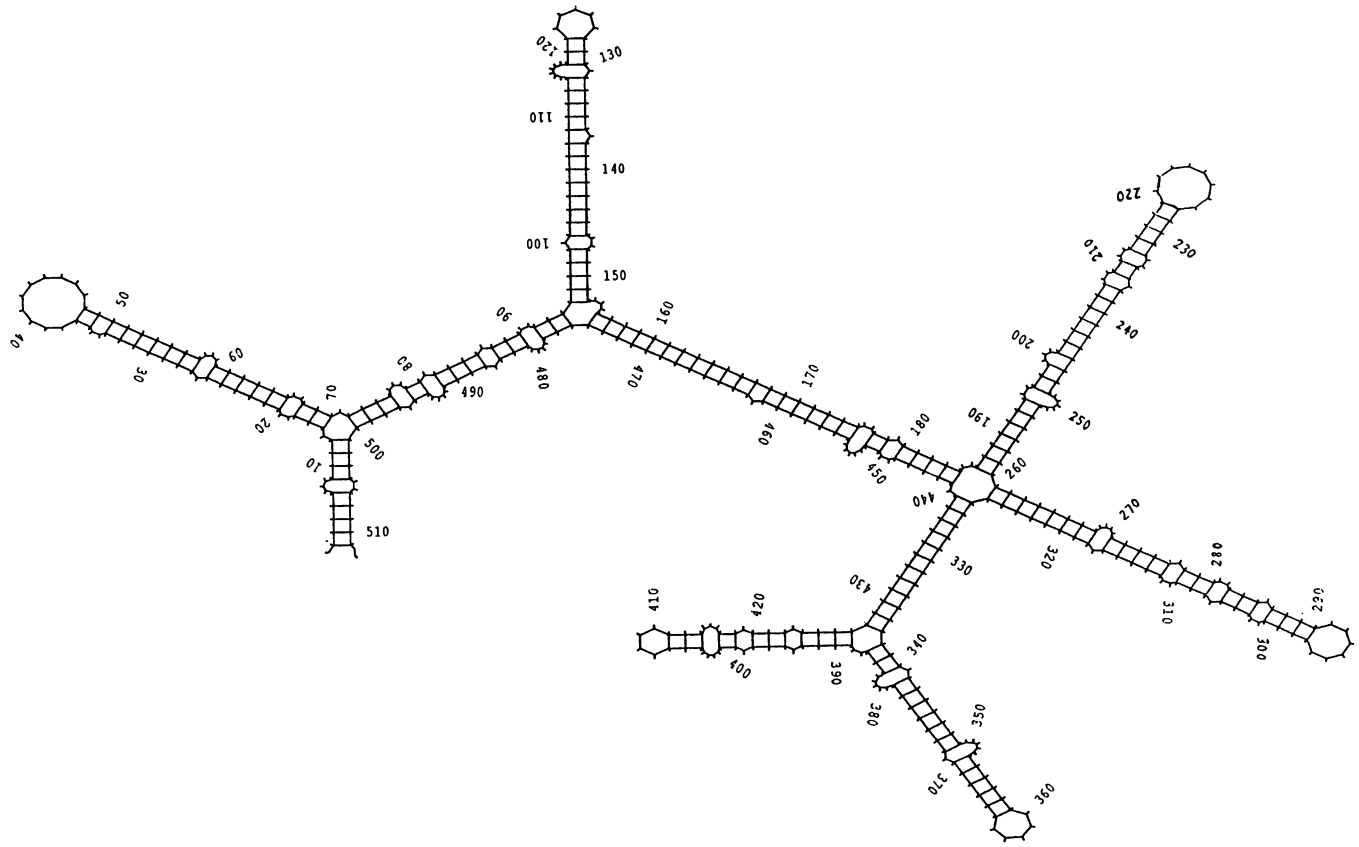

FIG. 3. Secondary structure predicted for the 5′ UTR of human IL-1RI mRNA. Secondary structure predictions were made by using the FOLD program as described. The fragment analyzed corresponds to all sequences of exon 1. Calculated free energy for the entire structure is $\Delta G = -255.4$ kcal/mol.

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CTCATTGGCAAAGTGAGCTG GTGGGCATAAGTGGGTTTTA AGTTTAAAAATTTAAAAACC -430

CTGTCTGCCCCCAAGTGTGG TATCAAGATTTTATAGTATG ACACTTAAATTGCTTTTTTC -370

                                          AP-1
ATCCGGGCGCGTAACAGCAA CAATGAAACCAGCAGATAAC GCGTGAGTAGTATCAGCTCT -310

GGGCCTGGCACTATTTTATA TGTATTAGCTCATTTTTTTT AAAAAACTGTTTTCAACCAC -250

    AP-1
TCCATGAGATGAGTGCTCTT ATGATCCCTTTTTCACAGAA GCGGAAACGGAGGTACAAAG -190

AAATTAGTGCACAAAGCCAG TCGGAGAGAGCCCCTGGCCA GGCACCAAGCTCCAGAGGTC -130

GCTCTGGCGAGCGTTTGCTT CGGGATCTGATGCCCTGGAG TGCCAAACTCAATTCGCGGT  -70

          SRE?
CGCAGCCAGGCTCCATGGGG GTAGTAGAGCCAGGTCGTAG TGGCTAGGTGAGTTGTCTCA  -10

          Sp1
ACTAACTCTAGTGGAGCCGC CGCAGCCCTGGAGGAGCCGG GCCAGCCGACTCGAGAGCGC  +51

                Sp1                                   Sp1
CCGGCAGCTCTCCAATGCTT TGGAACCGGCGGGACCCCTG CGGCTACCCGGGGCAGGGCG +111

GTGTCCGAGGGGTCTGTCCA GCCGCGCCTGCTCCTCGGTG GAGAGTGGAACCCGGCCAGC +171

                                      Sp1
TCGCTCGCAGCCCGCGACTG CCCAGCGAGCGTCTCGCCGT TCCCCGCCCCGCAGCGGCG +231

GCTAGAGCGAGACCGCGAAA GGCAGTTCCCGGCCGGAGGG CCGCAGCTTGTGGCCGGCGC +291

CGGAGCCGACTCGGAGCGCG CGGCGCGGCCGGGAGGAGCC GAGCGCGCCGGGCGCGGCGT +351

GGGGGCGCCGGCTGCCCCGC GCGCCCAGGGAGCGGCAGGA ATGTGACAATCGCGCGCCCG +411

CACCGTAGCACTCCTCGCTC GGCTCCTAGGGCTCTCGCCC TCTGAGCTGAGCCGGGTTCC +471
                                              Oligo 2

GCCCGGGCTGGGATCC
    BamH1
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FIG. 4. Nucleotide sequence of human IL-1RI putative promoter. The entire 976-bp *Eco*RI/*Bam*HI fragment (see Fig. 1*A*) is shown. The adenosine, which is the most 5′ transcription start site, is marked by an arrow and is numbered +1. Putative AP-1, Sp1, and SRE sites are boxed. A+T-rich regions are underlined. Oligo 2, used for primer extension, is underlined.

and the internal regulatory sequence (IRS), just 3′ of the initiation site, contain some common regulatory elements. There are two AP-1-like binding sites within the promoter, with the potential to bind AP-1 factors (20). However, the functionality of these elements within the IL-1R promoter remains to be tested. Within 250 bases downstream of the initiation site, there are four Sp1 or Sp1-like binding sites. Although the Sp1 sites studied to date are generally located upstream of the transcription initiation site, Sp1 can augment promoter activity at downstream locations (28). Also situated in the promoter is a SRE, and both SRE and AP-1 sites can respond to certain growth factors and tumor promoters, such as phorbol esters. In certain cell types, IL-1RI gene expression can be upregulated by phorbol esters and growth factors, such as IL-2, IL-4, and platelet-derived growth factor (7, 8, 10). It would be interesting to determine whether the IL-1RI promoter responds to these stimuli. We also found two 15- and 16-base-long A+T-rich domains in the promoter region, and similar A+T-rich sequences are thought to play a role in melting double-stranded DNA. Whether these two A+T-rich sequences play a role in opening two DNA strands during

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ACTCTA-G-TGGAGCCGC    IL-1RI
GCCCTC-ATTCTGGAGAC    Initiator (TdT gene)
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FIG. 5. Homology between the sequence surrounding the transcriptional start site of the IL-1RI and the initiator sequence of the terminal deoxynucleotidyltransferase (TdT) gene. Arrows, transcription initiation sites. Hyphens in IL-1RI sequence indicate two gaps inserted for alignment.

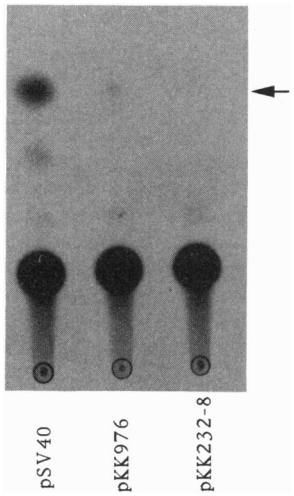

FIG. 6. Transient expression of CAT gene directed by the IL-1RI promoter. Human monocytic THP-1 cells were transfected with a plasmid bearing the bacterial CAT gene under the control of simian virus 40 early promoter (pSV40), IL-1RI promoter (pKK976), or no promoter (pKK232-8). CAT assays were performed as described. Equal amounts of cell lysate protein were used in each assay. Protein concentration in each lane was quantified by using a BCA protein assay kit (Pierce). Arrow, CAT activity (acetylation of [^{14}C]choramphenicol).

polymerase engagement at the onset of transcription is a matter of speculation (29, 30).

Of the mammalian genes identified so far, the promoters can be divided into three classes. A majority of genes are in the first class, having classic promoters that contain TATA or CAAT elements. A second class of promoters are those lacking the TATA and CAAT motifs, as well as a G+C-rich promoter region. Examples include genes that are regulated during mammalian immunodifferentiation, such as terminal deoxynucleotidyltransferase (31), the T-cell receptor β chain (32), and lck (33). A third class of promoters also lack typical TATA and/or CAAT sequences but are rich in G+C. The third class also includes those promoters that are not G+C-rich but contain multiple Sp1 sites. Eukaryotic genes containing this type of promoter usually encode enzymes with housekeeping functions. These include hypoxanthine guanine phosphoribosyltransferase (34), 3-hydroxy-3-methylglutaryl coenzyme A reductase (35), 3-phosphoglycerate kinase (36), and adenosine deaminase (37). In addition, promoters of several oncogenes and several growth factor receptor genes also belong to this category. They include c-Ha-ras (38), c-Ki-ras (39), human epidermal growth factor receptor (40), human insulin receptor (41), human insulin-like growth factor receptor (42), and nerve growth factor receptor (43).

These latter genes share some common characteristics: they have wide tissue distribution, a low constitutive level of expression, and generally have multiple transcription initiation sites. The promoter we have cloned for the IL-1RI also shares the features described above as indicated by primer-extension and CAT assay. Unlike IL-1α, IL-1β, and IL-1Ra genes, whose mRNAs are undetectable during normal physiological conditions, IL-1RI can be found at low levels in a wide variety of tissues and cell types. Our CAT assay in noninduced THP-1 cells agrees with this pattern of expression. We also observed a similar result in fibroblasts and YT cells (data not shown). Interestingly, CAT expression is inducible by phorbol ester only when sizable G+C-rich sequences were deleted (data not shown; unpublished data).

We found that sequences surrounding the transcription start site possess striking homology to an initiator sequence

(19) that appears to direct expression of a TATA-less gene. This sequence has been thought to bind transcription factor TFIID (44). More interestingly, Sp1 and AP-1 binding sites have been shown to interact with this initiator sequence to enhance the level of basal transcription. Our data strongly support the notion that the IL-1RI gene also belongs to this family. This finding provides insights into the regulation of IL-1R gene expression—i.e., what factors influence an increase or decrease in expression of the IL-1 receptor, as this may be an approach to downregulate the detrimental effects of IL-1.

We have not been able to localize the remaining transcription initiation sites in the genomic clone described here. Therefore, this raises the question whether the promoter we have cloned is the sole promoter of this gene or whether there are multiple promoters involved. According to the cDNA reported by Sims *et al.* (2), there are at least three IL-1RI mRNA isoforms, each cDNA obtained from different cell lines with a distinct 5′ UTR (John E. Sims, personal communication). However, they all share sequences of exon 2 (2). Our data of the primer-extension study are consistent with this finding. Oligo 1 derived from exon 2 generates four major bands, indicating that there are multiple species of mRNA that differ in sequences 5′ upstream of exon 2. Alternatively, this pattern might be due to intermediate pause sites during the reverse transcriptase reaction, considering the high G+C content of the template. However, we believe this is unlikely since we observed all four bands consistently in all cell lines studied. To further clarify this, oligo 2 derived from exon 1 was used and only one single band was observed. In this reaction, the annealing site is quite close to the previous one. This phenomenon is not cell-type dependent and we observed multiple primer-extension products in HepG2, THP-1, and fibroblasts (Fig. 2), as well as YT and Jurkat cell lines (data not shown). These data indicate that RNA polymerase II transcribes the different species of IL-1RI mRNA indiscriminately, but the effect of a different 5′ UTR on translation is currently unclear. The 5′ UTR derived from the human hepatoma (HepG2) mRNA (11) is relatively G+C-rich and constituted exon 1 in its entirety (514 bp; Fig. 1*A*), which makes up the majority of the 5′ UTR sequences of this class of IL-1RI mRNA. On the basis of computer analysis to generate secondary structure (ΔG = −255.4 kcal/mol), we hypothesize that this 5′ stem–loop structure on the IL-1RI mRNA may play a role in regulating mRNA translation, possibly by interference with ribosome scanning as suggested by Muller and Witte (45). Sequences with the potential to form stable secondary structures in the 5′ UTR of mRNA have been shown to inhibit translation both *in vitro* and *in vivo*. The free energy of the 5′ UTR of these mRNAs is even less stable than that of the IL-1RI (45–47). This is an attractive hypothesis as it adds another level of regulation to the IL-1RI.

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