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Structure and Transcription of the Mouse Erythropoietin Receptor Gene

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The complete gene encoding the mouse erythropoietin receptor was isolated by using a cDNA probe derived from a mouse erythroleukemia (MEL) cell library. The gene spans ~5 kilobases and is present in a single copy per haploid genome. It contains eight exons, and the nucleotide sequence of the coding region from the genomic DNA is identical to the sequence of one of the MEL cDNA clones except for a single amino acid substitution (Leu→Val) at codon 163. There is a cluster of three major transcriptional start sites ~150 nucleotides upstream of the initiator ATG codon which is conserved in erythropoietin-dependent and -independent erythroleukemic cells, in MEL cells at different stages of differentiation, and in normal bone marrow cells. The promoter region contains a potential binding site for Sp1, erythroid-specific transcription factor GF-1, and several CACCC boxes, but not typical TATA or CAAT sequences. A fusion gene containing 452 nucleotides of 5′ noncoding sequence linked to a promoterless human growth hormone gene directed the transcription of the latter in MEL cells but not in mouse fibroblasts, T cells, B cells, or macrophagelike cells, suggesting that this promoter functions in an erythroid-specific manner.

The principal regulator of erythropoiesis in mammals is the hormone erythropoietin (Epo). After binding to specific cell surface receptors, Epo stimulates the proliferation and differentiation of committed erythroid progenitor cells; this activity is associated with the activation of a number of erythroid-specific genes, including those encoding carbonic anhydrase and globin (2, 20). Epo receptors have been identified primarily on erythroid precursor cells, such as hematopoietic cell lines (3, 11–14, 26, 35, 41), splenic cells from mice treated with phenylhydrazine (29, 35) or infected with Friend virus (21, 35–37), and fetal liver cells (13, 31). More recently, Epo receptors have also been detected on megakaryocytes (11), suggesting that their expression may not be restricted exclusively to cells of the erythroid lineage.

Expression of the Epo receptor gene by hemopoietic cells represents one of the earliest events of commitment or differentiation to the erythroid lineage. Thus, an understanding of the regulation of this gene could shed light on early events of erythroid development. Experimental models of erythropoiesis have identified at least two types of Epo-responsive cells. An early cell type, called BFU-E, gives rise to large bursts of heme-containing cells and appears to be relatively insensitive to Epo (see reference 8 for a review; 13, 31). By contrast, a second cell type, called CFU-E, gives rise to smaller colonies of heme-containing cells that are highly sensitive to Epo and further advanced in erythroid differentiation. The dramatic acquisition of Epo sensitivity by these cells constitutes a major physiologic marker of early developmental events after commitment to the erythroid lineage. The molecular mechanisms regulating Epo responsiveness in these cells are unknown.

Cloning of the murine Epo receptor cDNA should facilitate the analysis of early events in erythroid differentiation (6). Two cDNA clones 1.75 and 1.86 kilobases (kb) in length (clones 190 and 141, respectively) were isolated from an expression library made from uninduced MEL cells. Each clone directed the binding and internalization of radioiodinated Epo. The nucleotide sequence predicted a polypeptide of 507 amino acids with a single membrane-spanning domain. There is significant homology to the human interleukin-2 (IL-2) receptor β-chain polypeptide (5, 15) as well as to the receptors for IL-4 and IL-6 (28, 43). Thus, these comparisons have revealed a new gene family of membrane receptors (5). Furthermore, expression of the Epo receptor cDNA clone in an IL-3-dependent lymphoid cell line allows the latter to grow in the presence of either IL-3 or Epo, indicating that the cloned receptor is also functional in vivo (22).

Unlike normal erythroid cells, MEL cells do not depend on exogenous Epo for proliferation and show different binding affinities for radioiodinated Epo (25, 36, 37). Since both Epo receptor cDNA clones were derived from this transformed cell line with Epo-binding properties and growth requirements different from those of normal erythroid cells, the possibility that these cDNAs were mutant forms of a normal receptor could not be excluded. We therefore sought to clone and characterize the normal gene encoding the mouse Epo receptor and analyze the transcriptional activity of its promoter. Our results should provide a basis for further developmental and structure-function studies of this receptor.

MATERIALS AND METHODS

Cell culture. MEL cells were cultured in suspension in Dulbecco modified Eagle medium (DME) plus 13% heat-inactivated fetal calf serum in a humidified CO_2 incubator

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(5% CO_2, 95% air) at 37°C (32). For monolayer cultures, cells were attached to 60-mm petri dishes precoated with fibronectin and induced with dimethyl sulfoxide for erythroid differentiation (32). EL4 cells (mouse T-cell lymphoma), S194 cells (mouse plasmacytoma), and WEHI-3 cells (mouse macrophagelike) were cultured in suspension in RPMI 1640; NIH 3T3 cells (mouse fibroblast) were cultured as monolayers in Dulbecco modified Eagle medium plus 10% calf serum; and HCD57 cells (Epo-dependent mouse erythroleukemia) were cultured in Iscove medium with 30% fetal calf serum, 5×10^{-4} M β-mercaptoethanol, and 0.5 U of human recombinant Epo per ml.

Isolation of genomic clones. A mouse T-cell partial *Sau*3A genomic library cloned in EMBL-3 (kindly donated by A. Winoto and D. Baltimore) was screened with the full-length *Kpn*I insert of clone 190 Epo receptor cDNA (6). The probe was labeled with ^{32}P by the random oligonucleotide primer method (10) and hybridized in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate [pH 8.3], and 1 mM EDTA), 5× Denhardt solution (1× Denhardt solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), and 0.2% sodium dodecyl sulfate for 16 h at 65°C. Positively hybridizing recombinant bacteriophage were plaque purified, and phage DNA was prepared as described previously (18).

DNA sequencing. Phage inserts were subcloned into the *Bam*HI site of the Bluescript vector (Strategene, Inc.), and subclones were generated either by cleavage at compatible restriction endonuclease sites or by progressive deletions with exonuclease III (16). Double-stranded plasmid DNA was purified by CsCl centrifugation and sequenced by the dideoxy-chain termination method (34), using Sequenase (U.S. Biochemical Corp.).

Nucleic acid analysis. For Southern analysis, genomic DNA was isolated from the liver of adult C129 mice, and 10-μg samples were digested to completion by one of several restriction endonucleases. After electrophoresis on 1% agarose gels, the DNA was transferred to Biotrans nylon membranes according to the suggestions of the manufacturer (ICN Biomedicals, Inc.). Filters were hybridized either with cDNA probes of specific activity 3.0×10^6 cpm/ml in a solution containing 50% formamide for 16 h at 42°C or with 5′-end-labeled oligonucleotide probes and washed at high stringency in 0.1× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate [pH 7])–0.1% sodium dodecyl sulfate for 1 h at 65°C.

Total cellular RNA was prepared by homogenization of tissues or cells in guanidinium isothiocyanate and centrifugation through CsCl (4). For primer extension analysis (24), an end-labeled oligonucleotide primer complementary to a sequence within exon 1 of the mouse Epo receptor (5′-CAGCAAGTAGGAGACAG; see Fig. 5) was synthesized and 5′-end-labeled with [γ-^{32}P]ATP. A 20-μg sample of total cellular RNA and 3×10^5 cpm of primer were incubated in annealing buffer [80% formamide, 0.4 M NaCl, 40 mM piperazine-*N*,*N*-bis(2-ethanesulfonic acid) (PIPES), (pH 6.4), 1 mM EDTA] for 16 h at 50°C and then precipitated with ethanol. Primer extension was performed for 1 h at 42°C in a buffer containing 50 mM Tris (pH 8.0), 10 mM $MgCl_2$, 40 mM KCl, 1 mM dithiothreitol, 1.25 mM each dATP, dCTP, dGTP, and dTTP, and 25 U of avian myeloblastosis virus reverse transcriptase. Samples of the reaction product were run on 5% polyacrylamide denaturing gels alongside known sequencing ladders. The probe for S1 nuclease protection was prepared by isolating a 0.52-kb *Ava*I-*Kpn*I fragment from one of the nested deletions that included 487 bases of 5′ noncoding sequence (see Fig. 4A and 5A). The *Kpn*I terminus, located within the polylinker of Bluescript, introduced five noncomplementary nucleotides into the corresponding position of the genomic DNA. After cleavage with *Ava*I and *Kpn*I, the reaction mixture was treated with calf intestinal alkaline phosphatase, and the desired band was isolated by

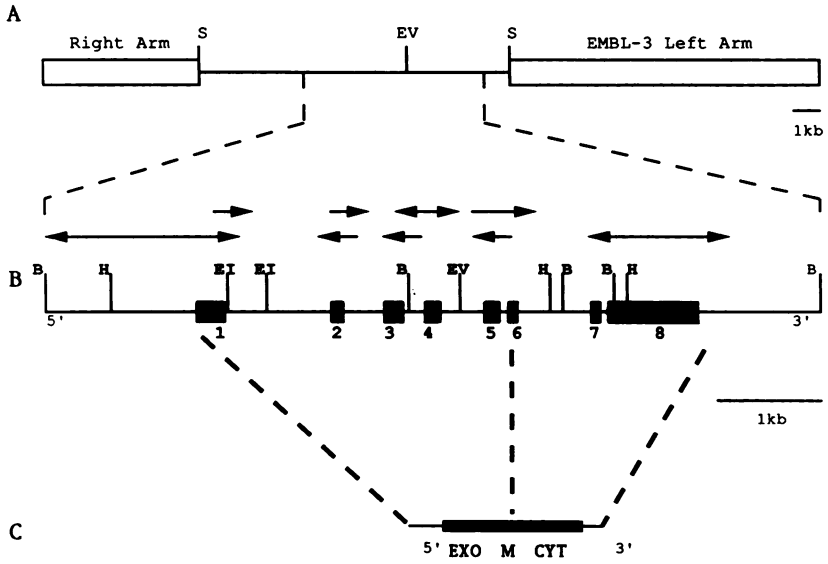

FIG. 1. Genomic organization of the mouse Epo receptor gene. (A) Schematic diagram of the bacteriophage λ11 clone. Only *Sal*I (S) and *Eco*RV (EV) sites are shown as landmarks. (B) Schematic diagram of the mouse Epo receptor gene, partial restriction map, and sequencing strategy. Arrows indicate the direction and length of the Bluescript subclones used for sequencing. The length and position of each exon are indicated (■). Restriction sites: B, *Bam*HI; EI, *Eco*RI; H, *Hin*dIII. (C) Relationship of the genomic DNA to the cDNA (——) and protein (▬). Different domains are demarcated by dashed lines. EXO, Exoplasmic domain; M, membrane-spanning domain; CYT, cytoplasmic domain.

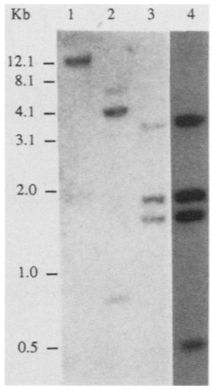

FIG. 2. Southern analysis of mouse DNA for the Epo receptor gene. DNA (10 μg) from normal mouse liver was digested with *Eco*RI (lane 1), *Hin*dIII (lane 2), or *Bam*HI (lane 3), and 4 ng of phage λ11 DNA was digested with *Bam*HI (lane 4). The filter was hybridized with the full-length insert of Epo receptor cDNA clone 190 (6). Locations of molecular weight standards are indicated at the left.

agarose gel electrophoresis. The fragment was end labeled with [γ-^{32}P]ATP and purified on a Sephadex column from unincorporated nucleotides. Total cellular RNA (10 μg) was incubated with 10^5 cpm of the probe after denaturation in annealing buffer at 50°C for 16 h. The S1 nuclease reaction was performed as described previously (24), using 50 to 75 U of enzyme (Bethesda Research Laboratories, Inc.) per reaction, and the products were resolved on a 5% sequencing gel.

Transient gene expression. The parent human growth hormone (hGH) expression vectors (39) and the hGH immunoassay system were obtained commercially (Nichols Institute). For the analysis of promoter activity, a *Dra*I-*Pvu*II fragment (−581 to −130; see Fig. 5A) from the 5′ noncoding region of the Epo receptor gene was ligated into the *Hin*cII site of p0GH, an hGH expression vector that lacks a eucaryotic promoter. The resulting plasmid (p0.45GH) contained the Epo receptor putative promoter 5′ to the cap site of the hGH gene and in the same transcriptional orientation. Another vector containing the herpesvirus thymidine kinase promoter upstream of the hGH gene in p0GH (pTKGH) served as a positive control (39). Supercoiled plasmid DNA (50 μg) was transfected into ~2×10^7 cells in suspension by electroporation essentially as described previously (33). Briefly, cells were washed twice in Hanks balanced salt buffer and chilled to 4°C for 10 min before electroshock (Bio-Rad Laboratories Apparatus) with 280 V and 960 μF. Medium was assayed for hGH 72 h after transfection by a radioimmunoassay as described by the manufacturer.

RESULTS

A total of 4×10^5 recombinant phage were screened with a full-length cDNA (clone 190) of the mouse Epo receptor, and five positively hybridizing clones were identified. By restriction analysis and hybridization with ^{32}P-labeled oligonucleotides complementary to different segments of the cDNA, the phage clones fell into two classes. One (λ11) contained the complete Epo receptor gene, whereas the others lacked the region from exons 1 to 3 (data not shown). All further results pertain to λ11. The 12-kb λ11 insert was subcloned into the Bluescript vector, and the nucleotide sequence corresponding to the entire coding region and parts of introns and flanking noncoding sequences was established (Fig. 1). The nucleotide sequence of the coding region was identical to that of clone 190 (4) except for two adjacent differences at positions 513 (C→G) and 514 (G→C) of the cDNA. This discrepancy predicts a Leu (in cDNA)-to-Val (in genomic DNA) substitution at codon 163 of the protein. Therefore, the normal genomic sequence is essentially identical with that of clone 190 (4).

Figure 2 shows a Southern analysis of mouse genomic DNA adjacent to λ11 DNA. The *Hin*dII- and *Eco*RI-digested patterns showed different junction fragments between genomic DNA and phage DNA because the λ11 clone is not

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                                        DONOR                    ACCEPTOR
                                     -----------------INTRON--------------
        ...EXON 1...AAA.G            GTAAGAACTGTA............CCCCTTTTCTAG
                    Lys Ala                           1.0  kb
                    38  39
 CG.GCC...EXON 2...CTC.GA            GTGAGTGAGTCC............CGATCCGCACAG
Ala Ala             Leu Glu                           0.40 kb
39  40              82  83
  G.GGT...EXON 3...GTA.G             GTAGGTGAGAGG............CACTTTCTGCAG
Glu Gly             Val Val                           0.24 kb
83  84              141 142
 TG.CTC...EXON 4...CAA.AGG           GTGAGAGCTAAG............CTCACCGCCCAG
Val Leu             Gln Arg                           0.42 kb
142 143             193 194
CTG.GAG...EXON 5...AGC.G             GTGAGGCCCTAG............CTTGCCTCCCAG
Val Glu             Ser Asp                           0.07 kb
195 196             245 246
 AC.CTG...EXON 6...CGC.CG            GTGAGCCCCTCA............CGATTTCAATAG
Asp Leu             Arg Arg                           0.76 kb
246 247             274 275
  G.GAC...EXON 7...TTC.CAG           GTAGGTAGGTGA............TTCTTTTTCCAG
Arg Thr             Phe Gln                           0.09 kb
275 276             303 304
CTG.TGG...EXON 8...TCC.TAG
Leu Trp             Ser
305 306             507
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FIG. 3. Intron-exon junctions of the mouse Epo receptor gene. The length of each intron, the sequence of intron-exon junctions, and the position of an intron with respect to the amino acid codon sequence are indicated.

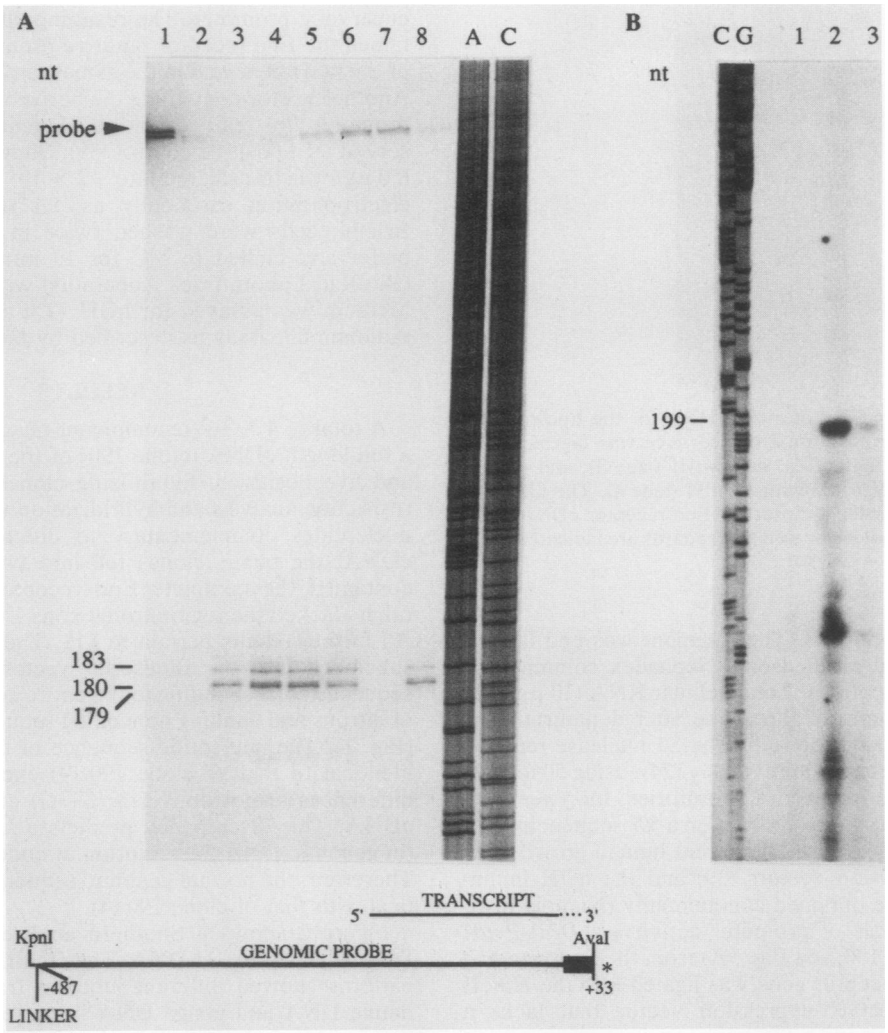

FIG. 4. Determination of transcriptional start sites of the mouse Epo receptor gene. (A) S1 nuclease protection: probe (lane 1); probe plus S1 nuclease (lane 2); uninduced MEL cell RNA (lane 3); day 2-induced MEL cell RNA (lane 4); day 4-induced MEL cell RNA (lane 5); HCD57 cell RNA (lane 6); normal mouse liver RNA (lane 7); normal mouse bone marrow RNA (lane 8); sequencing ladders used as size markers, reactions indicated above each lane (lanes A and C). The schematic diagram indicates the origin and length of the genomic probe. nt, Nucleotide; *, 5' end label; ■, segment of genomic DNA corresponding to the coding segment of exon 1. (B) Primer extension analysis using primer A (Fig. 5A): sequencing ladders used as size markers, reactions indicated above (lanes C and G); NIH 3T3 cell RNA (lane 1); uninduced MEL cell RNA (lane 2); normal mouse bone marrow RNA (lane 3).

long enough to include the distal *Hin*dIII and *Eco*RI sites of the genomic DNA. However, the *Bam*HI digest is particularly instructive, because *Bam*HI cleaves frequently in the region and thus allows a complete comparison of the bands between genomic and phage DNA digests. All fragments in the genomic DNA (Fig. 2, lane 3) were also observed in λ11 DNA (lane 4), strongly suggesting that the native gene was present in a single copy per haploid genome. The 0.5-kb *Bam*HI fragment, which is not seen in this autoradiogram of genomic DNA, was seen with longer exposure times (data not shown). The estimated length of the gene from the transcriptional start site (discussed below) to the polyadenylation site is ~5 kb. A schematic diagram of the gene and a restriction map are shown in Fig. 1. The extracellular domain is encoded by five exons, the transmembrane domain is encoded by a single exon, and the cytoplasmic domain is encoded by two exons, although primarily by exon 8. Starting at the transcriptional start site, exon 1 includes 5' noncoding DNA, the predicted signal peptide domain of 24 amino acids, and 14 amino acids of the mature protein. Exon 2 contains the only potential asparagine-linked glycosylation site in the extracellular domain. The membrane-spanning region is delimited by exon 6, while exon 7 may contain sequences that are involved in receptor-mediated signal transduction (see Discussion). The sequences of intron-exon junctions (Fig. 3) conform to consensus sequences of eucaryotic splice junctions.

At least three adjacent transcriptional start sites were identified by S1 nuclease protection (Fig. 4A) and primer extension (Fig. 4B). A cluster of three major protected fragments was identified by S1 nuclease analysis. Two of these sites appeared to be most prominent, although all were used in similar proportions in both normal bone marrow cells and two erythroid cell lines, Epo independent (MEL) one and the other (HCD57) absolutely Epo dependent for growth. In MEL cells, dimethyl sulfoxide-induced differentiation was not accompanied by any significant changes in the steady-state level of Epo receptor mRNA or in the

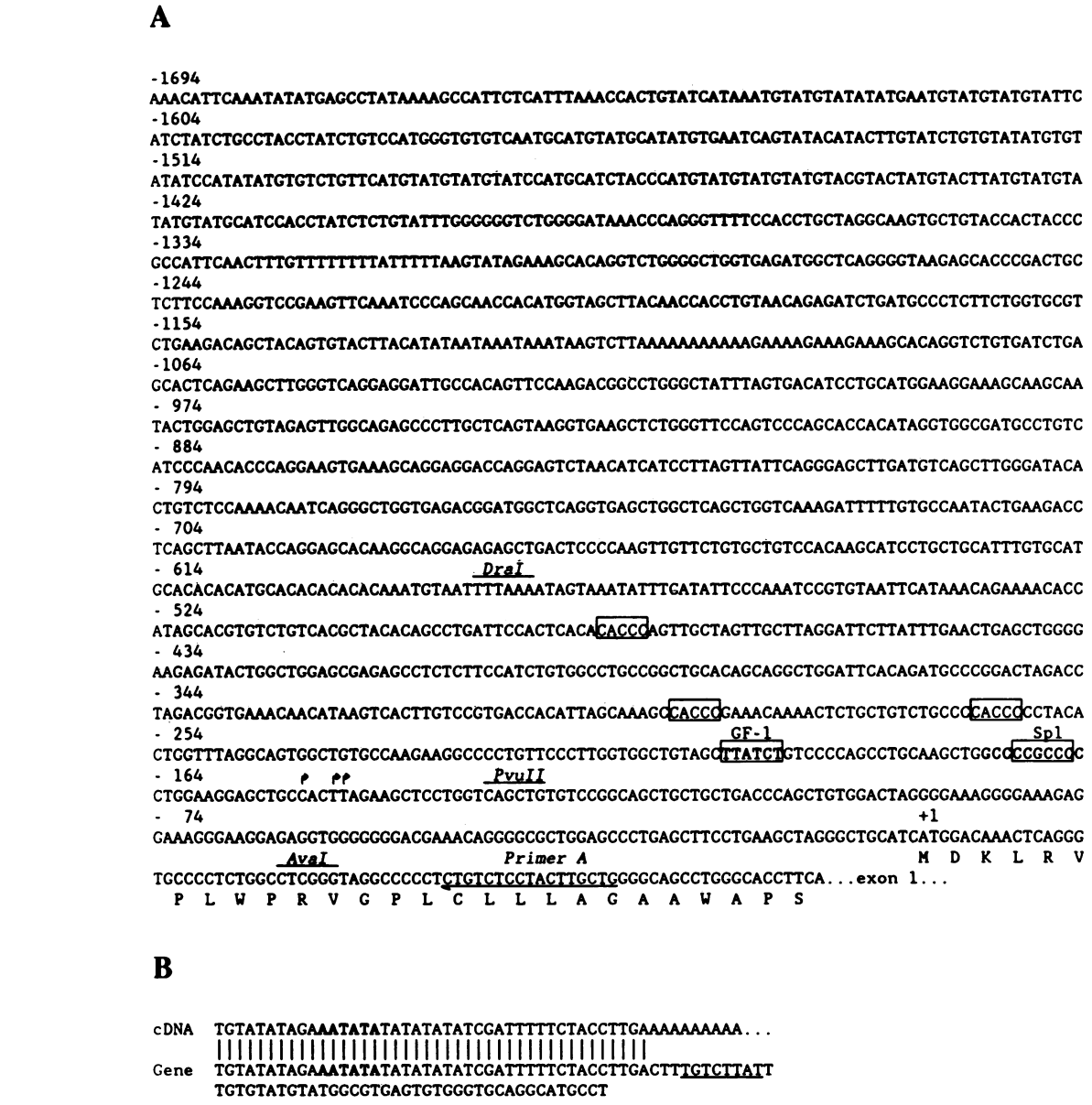

FIG. 5. Sequences of the 5'- and 3'-flanking regions of the mouse Epo receptor gene. (A) 5' upstream sequence and partial sequence from the coding region of exon 1 (4). Transcriptional start sites as defined by S1 protection (bent arrows), potential regulatory elements up to position −581 (box), relevant restriction sites, and the oligonucleotide primer used for primer extension (primer A) are indicated. (B) Comparison of the cDNA (4) and genomic sequences of the 3'-flanking region. The putative polyadenylation signal (bold) and a eucaryotic RNA-processing signal (underlined) are indicated.

selective utilization of cap sites (Fig. 4A). The largest protected fragment measures 183 nucleotides in length, beginning from the *Ava*I terminus in exon 1. This result assigns nucleotide C, located at −150 base pairs (bp) relative to the A of the initiation codon, as the initial transcriptional start site (Fig. 5A). Primer extension analysis with an oligonucleotide derived from exon 1 (primer A; Fig. 5A) yielded a maximum extension product of 199 nucleotides (Fig. 4B), which places the cap site at nucleotide 137, 14 bp downstream of the initial cap site identified by S1. The discrepancy between these two results is not clear, although it may be related to premature termination of the reverse transcript because of the presence of unfavorable mRNA secondary structure.

Sequences of the 5' and 3' noncoding regions are shown in Fig. 5. The overall nucleotide composition of the 5' noncoding region (1.67 kb) is 48% G+C, the content of the most proximal 5' region extending to the cap sites is ~61% G+C, and the content of the region from −1 to −580 is ~55% G+C. There are several long stretches of A+T in more upstream regions (Fig. 5A). There are no canonical TATA or CAAT boxes in the usual positions upstream of the cap sites. However, there is a potential Sp1-binding site (inverted complement of GGGCGG) beginning at position −166 (7), separated by about 22 bp from a potential site for the erythroid-specific transcription factor GF-1 or Eryf1 (inverted complement of [A/T]GATA[A/G]) (9, 42). A third motif with the core consensus sequence CACCC occurs three

TABLE 1. Activity of the EPO receptor gene promoter

Plasmid[a]	hGH production (ng/ml of culture medium)[b]				
	MEL	NIH 3T3	EL4[c]	SI94[c]	WEHI-3[c]
pTKGH	35 ± 3.6	46 ± 4.1	52 ± 1.5	>60	50 ± 14
p0GH	0	0	0	0.5 ± 0.1	0.3 ± 0.1
p0.45GH	10 ± 3.5	0	0	0	0.4 ± 0.1

[a] Plasmid constructs are described in Materials and Methods.

[b] Means and standard errors from four independent experiments. Values were obtained by plotting corrected counts per minute (minus background) onto a standard curve generated by using known hGH standards provided by the manufacturer (Nichols Institute).

[c] Cells grown in RPMI 1640 media. To avoid high concentrations of biotin, which competes for binding sites with a biotinylated anti-hGH antibody provided in the immunoassay kit, RPMI media were diluted eightfold before the assay and then corrected for the dilution factor when the final results were calculated.

times within 340 bp upstream of the cap sites boxed in Fig. 5. This site is also found in the promoter region of globin genes and has been shown to be of functional relevance (30).

The 3′-flanking region contains the hexanucleotide AATATA (Fig. 5B) upstream of the poly(A) tract in the cDNA. Although this sequence differs at the fifth position from the canonical polyadenylation signal (AATAAA), its location relative to the poly(A) tract and to a polypyrimidine consensus sequence in the genomic DNA that is required for efficient 3′-terminus processing (YGTGTTYY) (underlined in Fig. 5B) (27) suggests that it functions as a polyadenylation signal.

To determine the ability of the 5′ noncoding DNA to function as a promoter, a fusion gene containing nucleotides −581 to −130 from the ATG initiation codon of the Epo receptor gene (p0.45GH) was placed upstream of the promoterless hGH gene (plasmid p0GH) and transfected into either hematopoietic or nonhematopoietic cells (Table 1). Whereas p0.45GH directed transcription of the hGH gene in MEL cells, it was essentially inactive in EL4, S194, NIH 3T3, and WEHI-3 cells. The promoterless plasmid p0GH failed to show any significant transcriptional activity, whereas the pTKGH vector with the herpesvirus thymidine kinase promoter showed activity in all of the above-mentioned cell types. Thus, it appears that the 5′ noncoding segment from nucleotides −581 to −130 contains promoter sequences that are specifically active in erythroid cells.

DISCUSSION

The two Epo receptor cDNA clones isolated from a MEL cell library differed by a 2-bp deletion-insertion in a segment encoding the cytoplasmic domain, a discrepancy that led to the prediction of a different amino acid sequence for the 62 residues at the C terminus (6). Isolation of the full-length mouse Epo receptor gene has allowed us to determine the nucleotide sequence of the wild-type Epo receptor. Except for a neutral amino acid substitution, the sequence of the coding region from the genomic DNA is identical to that of cDNA clone 190.

The recombinant Epo receptor binds Epo specifically, and the ligand-receptor complex undergoes endocytosis (6). The site of ligand binding or the structural domains involved in signal transduction remain to be determined. Assuming that exons correspond to distinct functional domains or subdomains, a knowledge of the exon structure of this gene may be useful, for example, in the design of mutagenesis experiments to delineate different functional domains. The exoplasmic domain of the receptor is encoded by five different exons without any obvious repetitive structures. The membrane-spanning domain is encoded by a single exon. Interestingly, the majority of the cytoplasmic domain is encoded by a large single exon, exon 8. We suspect that this region of the receptor is involved in signal transduction. Finally, a small cluster of acidic residues in the cytoplasmic domain is present within 20 amino acids of the membrane-spanning domain. This region, encoded by exon 7, may harbor a signal for translocation. Similar motifs of clusters of acidic residues in the cytoplasmic domain, located approximately 20 amino acids from the membrane-spanning regions, have been noted previously in the low-density lipoprotein receptor, the transferrin receptor, the insulinlike growth factor II receptor, and the asialoglycoprotein receptor (23). All of these receptors undergo endocytosis but not degradation. It has been postulated that the acidic oligomer provides a signal for avoiding the degradation pathway after endocytosis. Although the postendocytotic fate of the Epo receptor is not yet fully known, the presence of this domain on a distinct exon leads to the prediction that the Epo receptor is also capable of circumventing the degradative pathway.

Acquisition of Epo sensitivity by erythroid progenitor cells appears to determine a critical step in erythrocyte development (8). Thus, the developmental regulation of the Epo receptor gene and, more specifically, analysis of its promoter may lead to the identification of unique *trans*-acting factors that mediate the transition from early BFU-E to later, Epo-sensitive stages. The promoter of the Epo receptor gene is moderately G+C rich and, as shown by our transfection experiments, exhibits erythroid cell specificity (Table 1). The activity of the promoter in megakaryocytes is not yet known, since most available megakaryocyte cell lines also show some erythroid characteristics.

The sequence of the promoter is also noteworthy in that it lacks TATA or CCAAT sequences in the vicinity of the cap sites, features that are generally attributed to tissue-specific genes (7). In this respect, the promoter sequence is similar to that of a number of other growth factor receptor genes, such as the insulin receptor (1), epidermal growth factor receptor (17), and nerve growth factor receptor (38). While none of these genes contain typical TATA or CCAAT motifs, the G+C contents of their promoters (73 to 88%) are significantly higher than that of the Epo receptor (55%). Other tissue-specific genes, including other erythroid-specific genes such as band 3 (19), also lack TATA or CCAAT motifs. Alternative elements needed for accurate transcriptional initiation of these genes have not yet been clearly defined, but the cap site itself may play a role (40). Other potential regulatory elements, including an Sp1-binding site (7), an erythroid-specific transcription factor GF-1-binding site (9, 42), and CACCC sequences found in β-like globin gene promoters (30), are also present. The fusion gene used for the transient expression analysis (p0.45GH) contains the Sp1 site, growth factor 1 site, and three copies of the CACCC element. It remains to be seen whether any of these elements are of relevance in the erythroid specificity of this particular promoter.

To our knowledge, the genomic structure of the Epo receptor is the first one in this superfamily of receptors to be characterized. It would be very interesting to extend this analysis to the IL-2 β-chain gene as well as to other members of this family as their cDNA clones become available.

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