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Immunology

Human interleukin 2 receptor β-chain gene: Chromosomal localization and identification of 5′ regulatory sequences

(promoter/Z-DNA)

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ABSTRACT **Interleukin 2 (IL-2) binds to and stimulates activated T cells through high-affinity IL-2 receptors (IL-2Rs). Such receptors represent a complex consisting of at least two proteins, the 55-kDa IL-2Rα chain and the 70-kDa IL-2Rβ chain. The low-affinity, IL-2Rα chain cannot by itself transduce a mitogenic signal, whereas IL-2 stimulates resting lymphocytes through the intermediate-affinity, IL-2Rβ receptor. We report here identification of the genomic locus for IL-2Rβ. The exons are contained on four *Eco*RI fragments of 1.1, 9.2, 7.2, and 13.7 kilobases. The 1.1-kilobase *Eco*RI fragment lies at the 5′-most end of the genomic locus and contains promoter sequences. The promoter contains no TATA box-like elements but does contain the d(GT)$_n$ class of middle repetitive elements, which may play an interesting regulatory role. The IL-2Rβ gene is localized to chromosome 22q11.2-q12, a region that is the locus for several lymphoid neoplasias.**

T lymphocytes play essential regulatory and effector roles in the immune response. A specific immune response is initiated through the binding of cell surface antigen receptors to peptide antigens presented in association with molecules encoded by the major histocompatibility complex. This binding initiates intracellular events, including the immediate release of intracellular second messengers (1), the induction of synthesis and secretion of the lymphokine, interleukin 2 (IL-2) (2), and the *de novo* expression of surface high- and low-affinity IL-2 receptors (IL-2Rs) (2, 3). IL-2 binding to the high-affinity IL-2R stimulates the events necessary for cellular proliferation. Expression of IL-2 and high-affinity IL-2R is transient and requires continued antigen receptor stimulation for a sustained immune response (2, 3). In addition to acting as a T-cell growth factor, IL-2 can stimulate activated B cells (4, 5), activated monocytes (6), natural killer cells (7), and large granular lymphocytes (8).

Three types of IL-2Rs have been identified. High-affinity ($K_d = 10^{-11}$ M) and low-affinity ($K_d = 10^{-8}$ M) receptors are expressed on the surface of activated T cells and human T-lymphotrophic virus type I-transformed T-cell lines (3). The intermediate-affinity ($K_d = 10^{-9}$ M) receptor is expressed at low levels on resting T cells and at somewhat higher levels on large granular lymphocytes (9, 10). The low-affinity IL-2R is composed of the IL-2Rα chain (Tac antigen, p55) and has a 13-amino acid cytoplasmic tail, presumably too short to mediate an enzymatic activity (11). The high-affinity IL-2R is composed of IL-2Rα and IL-2Rβ (p70) (12–14). Coexpression of these two chains yields high-affinity IL-2 binding, IL-2 internalization, and cellular proliferation. The β chain itself appears to constitute the intermediate-affinity IL-2R (12–14) and to be responsible for mediating high-dose IL-2 stimulation of resting lymphocytes and large granular lymphocytes, which generate lymphokine-activated killer cell activity (10). The deduced IL-2Rβ protein sequence (15) indicates a cytoplasmic domain large enough (286 amino acids) to potentially mediate signal transduction either by encoding an enzymatic activity or by coupling to other proteins. Therefore, the IL-2R system appears unique among multisubunit receptors in that two chains bind the same ligand independently, but in concert form an even higher-affinity receptor.

MATERIALS AND METHODS

Screening of cDNA and Human Genomic Libraries. A cDNA library was prepared in λ Zap II (Stratagene) from twice-selected, poly(A)-positive mRNA isolated from YT cells. The human genomic DNA library used was made in the laboratory of T. Maniatis (Harvard University) and was prepared by partial *Mbo* I plus *Hae* III digestion of fetal liver DNA and cloning into the *Eco*RI site of Charon 4A.

Oligonucleotides were synthesized corresponding to bases 38–131 (5′ probe) and 1788–1881 (3′ probe) of the IL-2Rβ sequence (15). Probes were labeled by random priming. Duplicate filters with a total of 200,000 phage from the cDNA library were differentially hybridized with the 5′ and 3′ probes, and three cDNA clones were selected based on hybridization to both probes. These clones were transduced into Bluescript plasmid vectors using the helper phage, R408 (Stratagene). Recombinant phage (10^6) were screened from the genomic DNA library through hybridization of duplicate filters with either the 5′ or 3′ probe. Hybridizing *Eco*RI fragments were subcloned into the pBluescriptII KS(−) plasmid vector (Stratagene).

Southern Blot Analysis. Total human genomic DNA from peripheral blood cells was digested to completion with *Eco*RI, *Bam*HI, *Pst* I, *Eco*RV, *Taq* I, *Sst* I, *Bgl* II, *Msp* I, *Hin*dIII, and *Pvu* II. Hybridization with radiolabeled probes was for 16–20 hr at 42°C in 50% formamide, 5× Denhardt's solution (1× = 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin), 5× SSC (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), and 0.1 mg of denatured salmon sperm DNA per ml. Membranes were washed twice in 2× SSC/0.1% SDS at 22°C and then twice in 0.2× SSC/0.1% SDS at 50°C.

DNA Sequence Analysis. The 1.1-kilobase (kb) *Eco*RI promoter fragment was subcloned into pBluescriptII KS(−) (designated pBS26) and into M13mp18 and M13mp19. Additionally, the 887-base-pair (bp) *Eco*RI–*Bal* I promoter fragment was subcloned into *Eco*RI- plus *Sma* I-digested M13 DNA. The plasmid pBS26 was also digested with *Alu* I, *Hae* III, or *Sau*3A and fragments were subcloned into *Sma* I- or *Bam*HI-digested M13. M13 subclones were identified by plaque hy-

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Abbreviations: IL-2, interleukin 2; IL-2R, IL-2 receptor; CAT, chloramphenicol acetyltransferase.

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                          -26                            1                                                       1965         2361
IL-2Rβ1 cDNA              5' CTGGGCTTTTAGTCTTTGCGGCCCCAGCAGCCAGAGCTCAGCAGGGCCCTGGAGAGATGGCCA.....TCCAAACCTC .....CGAATCCTCC 3'
                                                         1
Published IL-2Rβ sequence                             5' GCAGCCAGAGCTCAGCAGGGCCCTGGAGAGATGGCCA.....TCCAAACCTC.......CGAATCCTCC.....3'
                                                                                 21
IL-2Rβ2 cDNA                                                                  5' CCCTGGAGAGATGGCCA.....TCCAAACCTC 3'
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FIG. 1. Comparison of 5′ and 3′ endpoints of IL-2Rβ1 and IL-2Rβ2 cDNA clones with the published IL-2Rβ cDNA sequence (15).

bridization with the radiolabeled 1.1-kb *Eco*RI fragment and used for dideoxy DNA sequencing. The sequence‡ was prepared for publication using the DNA:DRAW program (16) on the National Institutes of Health Dec10 mainframe computer. DNA homology searches of GenBank version 60 were performed using the University of Wisconsin Genetics Computer Group package version 5 on a VAX computer located at the National Cancer Institute, Advanced Scientific Computing Laboratory, Frederick Cancer Research Facility.

Transfections and Chloramphenicol Acetyltransferase (CAT) Assays. The 896-bp *Sma* I–*Bal* I fragment was excised from pBS26 (using the *Sma* I site contained within the vector polylinker), *Hin*dIII linkers were ligated, and the fragment was subcloned in both orientations into the *Hin*dIII site of Jym-CAT-0, a vector containing the gene encoding CAT but not promoter or enhancer sequences (17). Transfections into YT or Jurkat cell lines with 5 μg of plasmid DNA were performed using DEAE-dextran as described (17). Cells were harvested 40–48 hr after transfection, and overnight CAT assays were performed with 0.2 μCi of [^{14}C]chloramphenicol (1 Ci = 37 GBq) and acetyl CoA (18 mg/ml) (17, 18).

Chromosomal Localization. DNA samples from 90 independent human–mouse or human–hamster somatic cell hybrids and subclones (19–21) were digested with *Eco*RI and the fragments were resolved on 0.7% agarose gels. Southern blots were hybridized with the radiolabeled IL-2Rβ1 cDNA insert and washed as above except that high-stringency washes were performed in 0.1× SSC/0.2% SDS at 55°C. The presence of the hybridizing human sequences in the DNA samples was correlated with the specific human chromosomes retained in each of the somatic cell hybrids.

In situ hybridization experiments were performed using peripheral blood lymphocytes from a normal male (46; XY) that were cultured for 72 hr at 37°C in RPMI-1640 medium supplemented with 15% fetal bovine serum, phytohemagglutinin (0.5 μg/ml), and antibiotics and then synchronized with BrdUrd (100 μg/ml) for 16 hr. Cells were washed and resuspended in fresh medium containing thymidine (2.5 μg/ml) and incubated for an additional 5.5 hr (22) with colcemid (0.05 μg/ml) present during the final 20 min. After centrifugation, swelling, and fixation, air-dried metaphase spreads were prepared (23). Chromosomal DNA was treated with RNase A (100 μg/ml) for 1 hr at 37°C and denatured for 3 min in 0.07 M NaOH in 64% ethanol (24, 25). Radiolabeled probe (specific activity = 3.2×10^7 cpm/μg) was prepared by nick-translation of IL-2Rβ1 plasmid DNA with [^{3}H]TTP and [^{3}H]dCTP and hybridized for 20 hr at 42°C. Slides were washed in 50% formamide/2× SSC (pH 7.0) for 10 min at 42°C and in 2× SSC at 42°C and then coated with a 50% solution of NTB2 nuclear track emulsion (Kodak). The slides were stored desiccated at 4°C for 9 days and then developed, stained (0.25% Wright stain), and photographed. The slides were then destained, and chromosomal banding was obtained by staining with 33258 Hoechst (150 μg/ml) for 30 min and exposure to UV illumination for 30 min after rinsing. The slides were again stained with Wright stain and the same metaphase spreads were rephotographed (22). Only metaphase spreads containing a grain on chromosome 21, 22, or Y were analyzed.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M32979).

RESULTS AND DISCUSSION

Although the gene encoding IL-2Rα has been characterized (11) and its regulation has been studied (17), it is clear that IL-2 responsiveness is mediated through the IL-2Rβ chain. We report here the identification of the IL-2Rβ genomic locus in order to begin to understand the regulation of IL-2Rβ gene expression.

Isolation of IL-2Rβ cDNA Clones. A YT cell-derived cDNA library was screened based on individual plaques hybridizing to 5′ and 3′ IL-2Rβ probes (see *Materials and Methods*) in order to select only full-length cDNA clones. Three independent clones were identified of 2.4 (IL-2Rβ1), 2.0 (IL-2Rβ2) (Fig. 1), and 1.8 kb (data not shown). Their apparent identity to the published cDNA sequence was confirmed by restriction enzyme mapping and partial sequence analysis (Fig. 1). The longest cDNA clone extended 26 nucleotides further 5′ than any IL-2Rβ cDNA described (15), corresponding to a minor transcription initiation site (see below).

Southern Blotting of Total Human Genomic DNA. Southern blots prepared from DNA isolated from four normal human donors were hybridized with the IL-2Rβ1 cDNA insert. After *Eco*RI digestion and hybridization, four bands of 13.7, 9.2, 7.2, and 1.1 kb were identified (Fig. 2*A*). Due to the apparent homogeneity of the four independent DNA samples and the lack of complexity of the various enzyme digestions (repre-

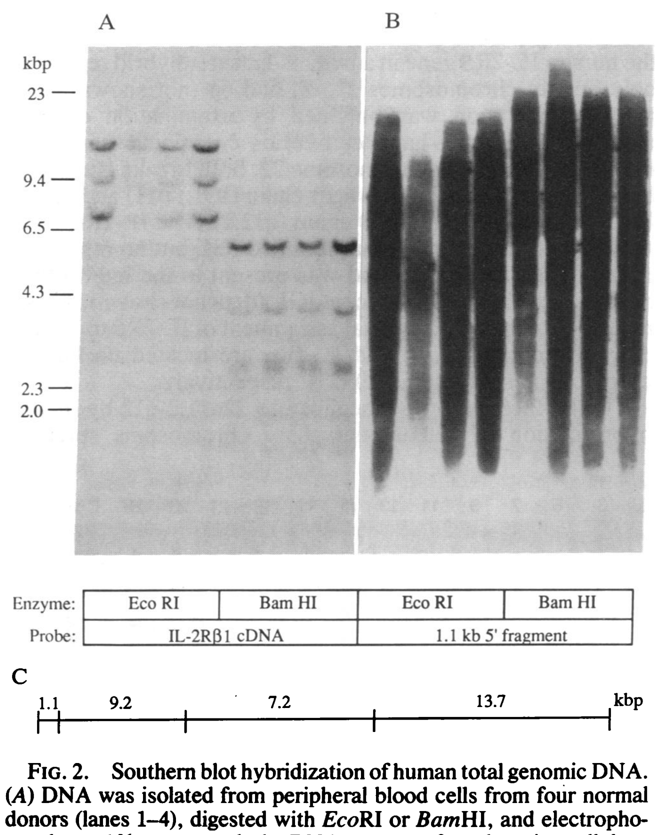

FIG. 2. Southern blot hybridization of human total genomic DNA. (*A*) DNA was isolated from peripheral blood cells from four normal donors (lanes 1–4), digested with *Eco*RI or *Bam*HI, and electrophoresed on a 1% agarose gel; the DNA was transferred to nitrocellulose and hybridized with the radiolabeled IL-2Rβ1 cDNA clone. (*B*) Reprobing of the blot of *A* with the radiolabeled 1.1-kb upstream *Eco*RI fragment shows uniform hybridization, suggesting it contains repetitive elements. (*C*) Genomic organization of the IL-2Rβ locus showing orientation of the four hybridizing *Eco*RI fragments.

sentative *Eco*RI and *Bam*HI digestions shown in Fig. 2*A* and data not shown), these data were most consistent with IL-2Rβ being encoded by a single or low copy number gene.

Isolation of Genomic Clones. The same oligonucleotide probes described above were used to screen a human genomic DNA λ library. In this case clones were picked based on hybridization to either the 5′ or 3′ probe. Five independent 5′ clones and two independent 3′ clones appeared unique based on *Eco*RI digestion patterns. Southern blotting of *Eco*RI digests from the various clones and hybridization with the IL-2Rβ1 cDNA insert identified all four *Eco*RI fragments that were detected in the human total genomic DNA Southern analysis (see Fig. 2*A*). Therefore, the entire gene was likely represented among the phage clones. The four hybridizing fragments were independently subcloned from the λ phage into the *Eco*RI site of pBluescript KS(−). Southern hybridization and partial sequence analyses identified the order of the four *Eco*RI fragments from 5′ to 3′ (Fig. 2*C*). It is possible that additional internal *Eco*RI fragments exist but do not contain coding sequences.

Chromosomal Localization. A somatic cell hybrid mapping strategy was used to localize the human IL-2Rβ gene. Southern analysis of DNA samples isolated from 90 human–rodent cell hybrids showed three hybridizing human bands (7.2, 9.2, and 13.7 kb) in *Eco*RI digests (Fig. 3), and all three bands segregated concordantly (i.e., either all present or all absent in any given somatic cell hybrid line), consistent with the interpretation that IL-2Rβ is a single copy gene. These bands were well resolved from cross-hybridizing bands in Chinese hamster DNA (2.8, 5.9, 8, 11, and 15 kb) and mouse DNA (3.1, 5.6, and 15.5 kb). Analysis of the entire panel of hybrids (Table 1) permitted unambiguous assignment of the gene to human chromosome 22. The gene segregated discordantly (≥19%) with all other human chromosomes. This chromosomal assignment was strongly supported by the presence of the human IL-2Rβ gene in a human–hamster hybrid retaining only human chromosomes 22, X, and 6p (not shown). Regional localization was obtained by examination of two independent human–hamster hybrids containing spontaneous breaks involving chromosome 22, both breaks occurring between immunoglobulin λ light chain (IGL, q11) and platelet-derived growth factor B chain (q12.1). The IL-2Rβ gene was absent in the hybrid that retained IGL but no other 22q marker (Fig. 3, lane 26) and was present in the hybrid that retained all 22q markers except IGL (data not shown). These results permitted the regional assignment of IL-2Rβ to 22q11-qter. In contrast, IL-2 and IL-2Rα are located on human chromosomes 4 (26) and 10 (27), respectively.

The IL-2Rβ gene was localized to 22q11.2-q12 by *in situ* hybridization of human metaphase chromosome spreads.

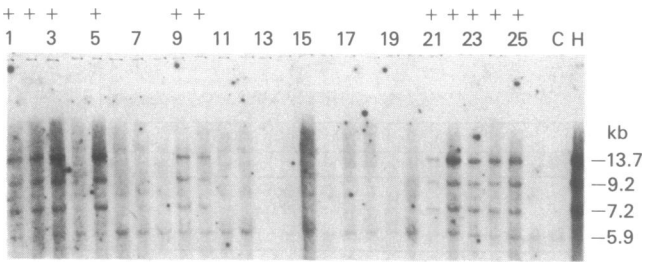

FIG. 3. Southern hybridization of representative human–hamster somatic cell hybrid DNA *Eco*RI digests with the IL-2Rβ1 cDNA probe. A different hybrid cell DNA was loaded in each numbered lane, and Chinese hamster (C) and human placental (H) DNAs are also shown. The sizes of the hybridizing human sequences (7.2, 9.2, and 13.7 kb) and a homologous hamster sequence (5.9 kb) are shown. Weakly hybridizing 8-, 11-, and 15-kb hamster fragments are poorly visualized. The presence of the hybridizing human sequences is indicated (+) above the lanes.

Table 1. Segregation of IL-2Rβ gene with human chromosome 22

Human chromosome	Gene/chromosome +/+	+/−	−/+	−/−	% discordancy
1	15	11	14	50	28
2	12	14	11	53	28
3	17	9	16	48	28
4	20	6	35	29	46
5	16	10	7	57	19
6	21	5	26	38	34
7	13	13	26	38	43
8	16	10	18	46	31
9	18	8	12	52	22
10	11	15	7	57	24
11	15	11	10	54	23
12	17	9	20	44	32
13	9	17	23	41	44
14	11	15	29	35	49
15	16	10	28	36	42
16	12	14	23	41	41
17	17	9	36	28	50
18	17	9	31	33	44
19	15	11	12	52	26
20	19	7	20	44	30
21	20	6	41	23	52
22	26	0	0	64	0
X	16	10	30	34	44

The human IL-2Rβ gene was detected as 7.2-, 9.2-, and 13.7-kb fragments in *Eco*RI-digested human–rodent somatic cell hybrids (see Fig. 3). Detection of the gene was correlated with the presence or absence of each human chromosome in the group of somatic cell hybrids. Discordancy represents presence of the gene in the absence of the chromosome (+/−) or absence of the gene despite presence of the chromosome (−/+), and the sum of these numbers times the total number of hybrids examined (×100) represents % discordancy. The human–hamster hybrids consisted of 28 primary hybrids and 12 subclones (17 positive of 40), and the human–mouse hybrids represented 16 primary clones and 34 subclones (9 positive of 50).

Given the data from somatic cell hybrids that the IL-2Rβ gene mapped to chromosome 22, only metaphase spreads containing hybridization to small chromosomes (i.e., 21, 22, and Y) were examined. Fifty-eight percent (19/33 total) of the grains on chromosome 22 were localized to this region (Fig. 4). In contrast, there was a random distribution of grains on chromosomes 21 and Y representing nonspecific background. An additional 71 grains were randomly distributed on the other 20 autosomes (data not shown).

Several neoplasias of hematopoietic origin have been associated with nonrandom aberrations involving human chromosome 22 (28). Based upon its chromosomal location and function, IL-2Rβ must be considered as a potential candidate gene in any of these neoplasias, and genetic linkage analysis would facilitate this evaluation. For this purpose, DNA samples from 10 unrelated individuals were evaluated for the presence of restriction fragment length polymorphisms (RFLPs) identified by the IL-2Rβ probe. No RFLPs were detected after DNA digestion with *Eco*RI, *Hin*dIII, *Bam*HI, *Msp* I, *Taq* I, *Eco*RV, *Pst* I, *Sac* I, *Bgl* II, *Xba* I, and *Pvu* II. In *Kpn* I digests, a 5.2-kb band was detected in 18 of 38 individuals tested and invariant bands of 12 kb and 31 kb were also present (data not shown). Presumably one allele was not resolved from one of the constant bands in *Kpn* I digestions of DNA from individuals not containing the 5.2-kb band. Moreover, it is highly probable that the $d(GT)_{26}$ tract in the 5′ flank of the IL-2Rβ gene (see below) is polymorphic (29, 30). Amplification of this tract using the polymerase chain reaction with flanking oligonucleotide primers could make this locus highly informative (29, 30).

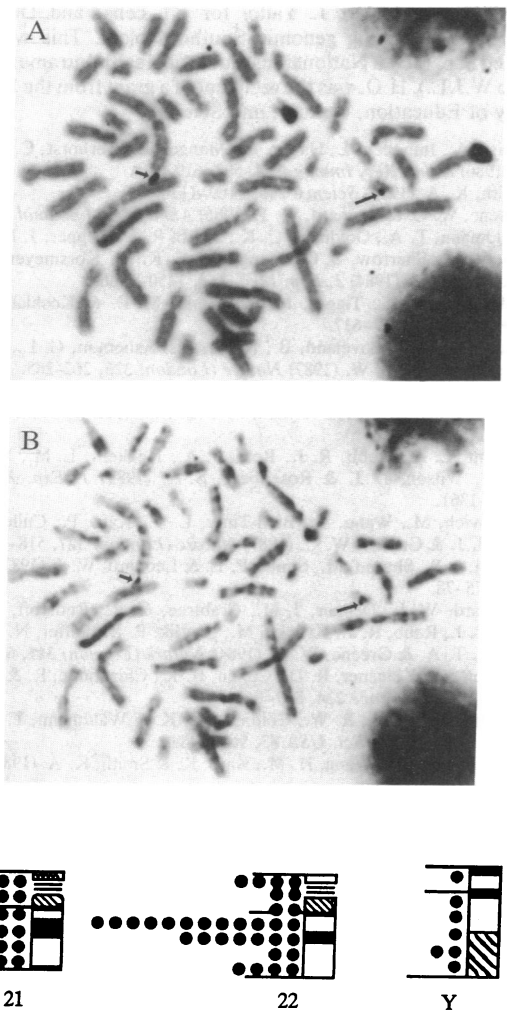

FIG. 4. *In situ* hybridization. (*A*) Representative metaphase spread containing a grain on chromosome 22q11 (long arrow) and a background grain on chromosome 2 (short arrow). (*B*) The metaphase spread of *A* after chromosome banding. (*C*) Distribution of grains on chromosomes 21, 22, and Y in 47 metaphase spreads.

Identification of the IL-2Rβ Gene Promoter. Since the 1.1-kb *Eco*RI fragment contained sequences upstream of the 5′ end of the cDNA, it was hypothesized to contain promoter sequences. The fragment was sequenced (Fig. 5) and found to contain 124 bp corresponding to the 5′ end of the IL-2Rβ1 cDNA and 143 bp apparently corresponding to an intron. A canonical splice donor site is present at the indicated exon–intron boundary. Interestingly, the ATG translation start site (15) is not contained within the first exon. Transcription initiation sites were mapped by S1 nuclease digestion and primer extension. S1 digestion showed that the major transcription initiation site mapped to the end of the cDNA clone identified by Hatakeyama *et al.* (15) (designated +1 in Fig. 5; data not shown) and a single primer extension experiment showed another, minor transcription initiation site that corresponded to the 5′ end of IL-2Rβ1 cDNA clone (denoted at position −26 in Fig. 1; data not shown). Examination of the upstream sequences showed no TATA box or CAAT box sequences. Two AP-2 sites (consensus sequence of $\begin{matrix}T\\C\end{matrix}\begin{matrix}C\\G\end{matrix}\begin{matrix}C\\C\end{matrix}\begin{matrix}C\\C\end{matrix}\begin{matrix}A\\C\end{matrix}\begin{matrix}N\\C\end{matrix}\begin{matrix}G\\G\end{matrix}\begin{matrix}C\\C\end{matrix}$, where N = unspecified nucleotide) (32) at −796 to −787 and −298 to −289 and one SP-1 site (CCGCCC; ref. 33) at −163 to −158 (all boxed in Fig. 5) were identified. A second canonical SP-1 site was identified downstream of the major transcription initiation start site (+79 to +84).

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GAATTCATGG AAATGGGAAG GGCAGTGATG GAGATGGGAA GGGCAGTGGG   -804

                AP-2
GGTTGGAGGG GTGGGGATTG TTGTTGGGTA CGAAAACAGA ATTAGATCGA   -754
ATGAATAAGA TCTAGTATTT GATAGCATAA CAGGGTGACT TTAGTCAACA   -704
ATAATTTATT GTACATTTAA AAATAACTAA AAGAGTATAC TTGGATTTTA   -654
ACACAAAGAA AGGATAAATA CTTGAGGTGA TGGATACCCA TTACCTGATG   -604
TGATTATTAT ACATTGTATG CCTGTATCAA AATAGCTCAT GTGCCTCATG   -554
AATATAGACA CCTACCACAT GCCCACAAAA TTAAAAACTA AAAAAAACAG   -504
TCATCTCTGA ATGCTAAACG GAGTAAGGGG CTTCCTGGAA GGCTGGGTGA   -454
AATGGGAGTC TCGGAAAGAT GGTGTGTTGC AGGCTGGGAG GAGGGTGAGA   -404
CGCTGGGGTC ACCTAGAGGG ACCTGCTTGT GTGAAGCCTA CGTATTAGTG   -354
GGTATGTGTG TGACCGGATG GAGGCGTCAG AGGTGTTGGG TAGCCTGTGT   -304

          AP-2
GAGTTGGGCGT GGGGGTGATG TAGGAGGGGA GAGAGGGAGG GCCTGCGTTC   -254
CCTTGGCTCC TGTGTGCAGC TAGGCCCCTA TTTGACAATG TGTGTGTGTG   -204

                                                SP-1
TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG CCGCCCCCAG   -154
CGTAGGAGGC AGATCTTTAT CTGGCCCTGG GTGCTTGAGG AGTTTCAGGC   -104
TTTCTCATAA GCCTCGTCTC CCCGAATCTC CACCCCAGCC TTGCCCCTCT    -54

                          -26
ATCCTCTGCA CAGGAAGTGG GCTGGCTCTG GGCTTTTAGT CTTTGCGGCC     -4

+1
CCAGCAGCCA GAGCTCAGCA GGGCCCTGGA GAGATGGCCA CGGTCCCAGC    +47
ACCGGGGAGG ACTGGAGAGC GCGCGCTGCC ACCGCCCCAT GTCTCAGCCA    +97

Beginning of first intron
GGTGATGTCC CCCTGCCTCC TCCCGGCCCC TGTGGACAGC CAGAGGGCTG
GGAGTGAAAG TCACAGAGAA GACTTTCAGC TCTGACTCAG TTCCCCCAGC
AGTTTCTGCC TGAACTCCCA TCCCCCAACT TTGTCTTAGA ATTC
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FIG. 5. Nucleotide sequence of the 1.1-kb upstream *Eco*RI fragment of the IL-2Rβ gene. Nucleotide numbering is based on the major transcription initiation site (+1). Two AP-2 sites (−796 to −787 and −298 to −289) and one SP1 site at −163 to −158 are indicated by boxes. A second SP-1 site is 3′ of the major transcription initiation site (+79 to +84). An AP-1-like sequence (CGTCA) (31) is located at −329 to −325. The $d(GT)_{26}$ Z-DNA sequences are italicized, and sequences homologous to the alcohol dehydrogenase gene π subunit and $\sigma_{\gamma 3}$ region 5′ flanking sequences are underlined.

Although the SP-1 sites studied are generally located upstream of transcription initiation, SP-1 can augment activity from downstream locations (34). An AP-1-like, 5-nucleotide sequence previously identified as important in c-*fos* gene expression was also noted at −329 to −325 (CGTCA) (31). The IL-2Rβ upstream region also contains a stretch of 52 bases containing purine-pyrimidine repeats (italicized in Fig. 5). A d(GT) repeat of such length represents a family of middle repetitive elements present at $\approx 10^5$ copies in the human genome (35). This was confirmed by probing restriction enzyme-digested, total genomic human DNA with the 1.1-kb *Eco*RI fragment and seeing uniform hybridization (Fig. 2*B*), indicative of hybridization to repetitive elements. As expected, comparison of this sequence to those in GenBank revealed similarity to many genes containing the $d(GT)_n$ stretches. Interestingly, a $d(GT)_{26}$ repeat may be expected to form a Z-DNA structure, and similar sequences have been shown to exhibit enhancer-like function (35–37). This may have implications for the means of regulation of expression of IL-2Rβ. In addition to the $d(GT)_n$ repeat, GenBank searches revealed two matches: (*i*) an 86% homology between −755 and −657 of IL-2Rβ and sequences contained in the 3′ untranslated region of the class II enzyme of human liver alcohol dehydrogenase π subunit mRNA (38) and (*ii*) a 75% homology between −722 and −618 of IL-2Rβ and 5′ flanking sequences of the $\sigma_{\gamma 3}$ region that is contained within the human immunoglobulin heavy chain constant region gene cluster (39). Although the extent of these overlapping homologies is highly significant, the function of this sequence is unclear and will require further studies.

Without classical CAAT or TATA box sequences it was critical to demonstrate that sequences within the 1.1-kb *Eco*RI fragment contained promoter activity. This was done by subcloning the *Sma* I–*Bal* I fragment from pBS26 upstream of

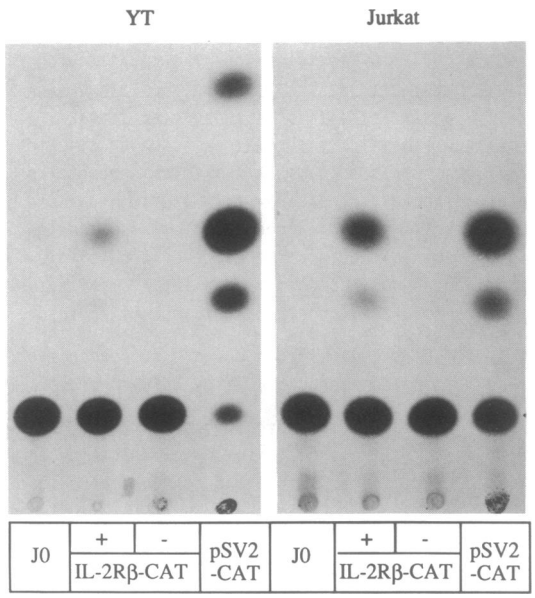

FIG. 6. Sequences 5′ of the IL-2Rβ gene have promoter activity. The 896-bp *Sma* I–*Bal* I fragment was subcloned in + and − orientations upstream of the CAT reporter gene and used in transient transfections of YT or Jurkat cells. The vector pSV2CAT containing the simian virus 40 promoter and enhancer (18) served as a positive control for transfections, whereas the vector Jym-CAT-0 (J0) served as a negative control for vector sequences. Percent conversions of chloramphenicol to the acetylated form for YT cell transfectants were as follows: J0, 0.65%; IL-2Rβ(+)-CAT, 3.4%; IL-2Rβ(−)-CAT, 0.34%; pSV2-CAT, 94%. Percent conversions for Jurkat cell transfectants were as follows: J0, 0.17%; IL-2Rβ(+)-CAT, 8.9%; IL-2Rβ(−)-CAT, 0.61%; pSV2-CAT, 35%.

the CAT gene. The *Bal* I site is located in the first exon, thus retaining the transcription initiation sites but eliminating a portion of the contained cDNA sequences and the exon–intron junction. This fragment in the correct orientation, but not reverse, was able to drive expression of the CAT reporter gene (Fig. 6). The promoter fragment was active in YT cells, which express IL-2Rβ, but also had significant activity in Jurkat cells, which express no detectable IL-2Rβ. Thus, additional sequences, either 5′ or 3′ or within the body of the gene, may serve an important role in controlling expression.

A number of genes whose products serve "housekeeping" functions and are expressed constitutively do not contain TATA sequences. This is consistent with constitutive expression of IL-2Rβ protein (10, 40) and mRNA (15) in resting T lymphocytes. However, IL-2Rβ mRNA levels do increase after T-cell activation (ref. 15; unpublished results), indicating an inducible regulation of expression. The nucleotide consensus sites identified (SP-1, AP-2, and the limited AP-1-like sequence) may play a role in such regulation. The level of IL-2Rβ regulation differs markedly, however, from that shown for IL-2Rα gene expression. IL-2Rα is an activation antigen: expression is not detected at the mRNA or protein level in resting T cells but is greatly induced after antigen stimulation (3). Therefore, the regulation of expression of IL-2R proteins is in keeping with the apparent functions of the individual subunits. IL-2Rα expression is important in the acute phase of an antigen-stimulated immune response, which is transient in nature, and may modulate (increase) the affinity of the IL-2R for its ligand, whereas IL-2Rβ expression is necessarily constitutive since that polypeptide appears important for IL-2-mediated signaling and activation without antigen specificity. Future studies are necessary to address the coordinant and discordant means of regulation of these separate genes.

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