Volume 13 Number 21 1985 Nucleic Acids Research

Molecular cloning and structure of the human interleukin 2 receptor gene

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Received 9 September 1985; Accepted 15 October 1985

ABSTRACT

We have cloned the IL-2 receptor gene from human genomic DNA libraries using IL-2 receptor cDNA as probe. The genomic DNA segments that hybridized with cDNA were subcloned in M13 phages and their sequences were determined. The nucleotide sequences showed that the IL-2 receptor gene was encoded by eight exons and that the coding region sequences agreed completely with that of the IL-2 receptor cDNA cloned from a cell line derived from adult T cell leukemia (ATL), in which IL-2 receptors are expressed abnormally. The nucleotide sequence of the 5'-flanking region had a putative promotor region, which had some homology with the human IL-2 gene. Transcription initiation sites were clustered about 25 bp 3' to the TATA box as assessed by primer extension analysis. These sites for normal and ATL T cells were the same. Exons 2 and 4 encoding the extracytoplasmic portion had significant homology, suggesting that the two exons are derived by duplication of an ancestral exon. Exon 2 contained six cysteine residues, four of which are conserved at the corresponding positions in exon 4.

INTRODUCTION

Molecular genetic studies have shown that DNA rearrangements and point mutations contribute to immunoglobulin diversity (1-3). DNA rearrangements were also shown to be involved in the generation of the genes encoding the T cell antigen receptor (4). Although such random genetic events greatly amplify the otherwise limited germline diversity of lymphocytes, such events also sometimes produce byproducts that may react self-components. Selection mechanisms are needed to counteract such auto-reactive lymphocytes. Peripheral T cells stimulated by specific antigens proliferate and consequently expand their progenies. The T cell proliferation is regulated by the interaction of a lymphokine, interleukin 2 (IL-2) and its receptor (5). Both IL-2 and its receptor genes are transcribed only in activated T cells after antigen stimulation.

An excessive number of IL-2 receptors are constitutively expressed on leukemic cells or cell lines derived from adult T-cell leukemia (ATL) (6,

7) that are infected by the human-C-type retrovirus HTLV-I/ATLV (human T-cell leukemia virus/adult T-cell leukemia virus) (8, 9). The aberrant expression of this growth factor receptor may be closely associated with the leukemogenesis of ATL (10, 11). As a first step to understand the molecular mechanisms of normal and aberrant regulation of the IL-2 receptor gene, we have cloned human and mouse IL-2 receptor cDNA and studied their primary structures elsewhere (12,13). Human receptor cDNA clones have also been isolated by other groups (14,15). The IL-2 receptor gene is neither grossly rearranged nor does the size of its mRNA differ in normal and ATL cells.

Here, we cloned and characterized genomic DNA encoding the human IL-2 receptor. The complete nucleotide sequences of eight exons from normal tissues ruled out the possibility of the any point mutations in the IL-2 receptor cDNA isolated earlier from ATL cell line MT-1. The nucleotide sequence of the 5'-flanking region showed an interesting promotor structure; we identified the sites where transcription was initiated.

MATERIALS AND METHODS

Materials - The restriction endonucleases, T4 ligase, reverse transcriptase, T4 polynucleotide kinase, and an M13 sequence kit were purchased from Takara Shuzo, Co., Ltd. or from Toyobo Co., Ltd. α-^{32}P-dCTP (3000 Ci/mmol) was obtained from Amersham and New England Nuclear.

Cloning of recombinant phages - The Charon 4A phage library containing AluI-HaeIII partial digests of human fetal liver DNA we used was the generous gift of T. Maniatis (16). A Charon 4A phage library containing EcoRI partial digests of human placental DNA was made by N. Takahashi (unpublished). Phages were screened as described elsewhere (17), using the 1.8-kb PvuII-HindIII fragment (probe a) of the IL-2 receptor cDNA as probe (12).

Nucleotide sequence determination - Cloned phage DNA was digested by restriction enzymes and fragments containing exons were identified by Southern blot hybridization with IL-2 receptor cDNA as probe (18). Such fragments were isolated, digested with other restriction enzymes, and ligated with one of the M13 phage vectors (mp10, mp11, or mp18) (19). The recombinant DNA was used for transfection of Escherichia coli JM103. The phage containing an exon sequence were screened for by plaque hybridiation using IL-2 receptor cDNA as probe (17). The positive M13 phage clones were sequenced by the Sanger method (19, 20).

Primer extension - A 17-base primer complementary to the sequence shown in Fig. 3 was synthesized by an automatic DNA synthesizer (Applied Biosystems, Model 310). The primer was phosphorylated by T4 polynucleotide kinase and annealed to 5 µg of mRNA prepared from various cells. The samples were reverse-transcribed and loaded on 8M urea gel as described before (21).

RESULTS AND DISCUSSION

Cloning of human IL-2 receptor gene -- Phage libraries containing human placental or fetal-liver DNA were screened using a human IL-2 receptor cDNA fragment (probe a in Fig. 1) as probe. Out of 3 x 10^6 phages in the

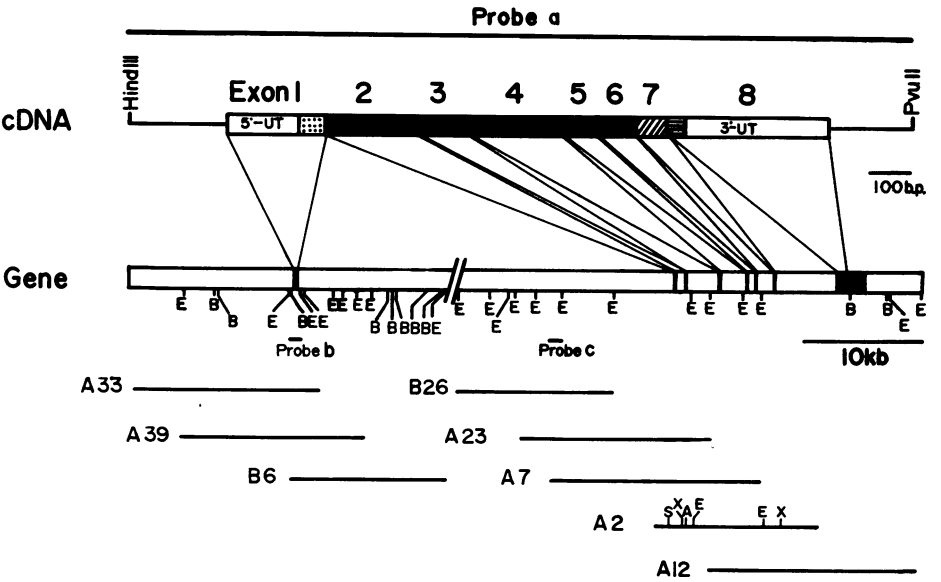

Fig. 1. Organization of the human IL-2 receptor gene. Upper and lower rectangles indicate cDNA and genomic DNA of the IL-2 receptor, respectively. Dotted, closed, hatched, and horizontal rectangles in cDNA indicate a signal peptide, extracytoplasmic region, transmembrance region, and intracytoplasmic region, respectively. Closed rectangles in gene show exons. Locations of exons were determined using marker restriction sites or regions surrounded by restriction sites as follows; exon 1, PstI; exon 2, 1.1-kb SacI-XbaI region; exon3, 0.5-kb ApaI-EcoRI region; exon 4, PstI; exon 5, StuI; exon 6, AatII; exon 7, 1.5-kb EcoRI-XbaI region; exon 8, BamHI. these sites are shown in Fig. 1 (clone A2) and Fig. 2. Horizontal lines at bottom indicate isolated Charon 4A phage clones. A and B series were isolated from the library of AluI-HaeIII partial digests and from that of partial EcoRI digests, respectively. E, EcoRI; B, BamHl; S, SacI; X, XbaI; A, ApaI. Probe a, HindIII-PvuII fragment of cDNA (1.8 kb); Probe b, the 5'-EcoRI fragment of B6 (0.7 kb); Probe C, EcoRI fragment of A7 (1.3 kb); UT, untranslated region.

libraries, four positive clones (A2, A7, A12 and B6) were found. Cleavage mapping with restriction enzymes and Southern blot hybridization indicated that many of the clones overlapped each other, as shown in Fig. 1. B26 and A23 clones containing the regions 5' to clone A7 were isolated using the 1.3-kb EcoRI fragment of A7 as probe (probe c). These two clones did not contain any fragments overlapping with clone B6. A33 and A39 clones were isolated using the EcoRI fragment of B6 clone closest to the 5' end (probe b) as probe.

As described elsewhere (12), three EcoRI fragments (10, 6, and 2.4 kb) and two BamHI fragments (30 and 7 kb) of human placental DNA hybridized to IL-2 receptor cDNA. Here, when all of the genomic clones were digested with EcoRI or BamHI and transferred to nitrocellulose filters, five EcoRI fragments (10, 6, 2.4, 1.3, and 1.1 kb) and a BamHI fragment (7 kb) hybridized with the IL-2 receptor cDNA, which mostly agreed with the earlier results. The smallest two EcoRI fragments might have been overlooked before because they were not efficiently transferred to the filters. There must be another BamHI site in a uncloned region, which is required to account for the 30-kb fragment.

Nucleotide sequence determination of the IL-2 receptor gene -- Nucleotide sequences of exons and their flanking regions were determined according to strategies shown in Fig. 2. The sequences showed several interesting features of this gene (Fig. 3). First, the IL-2 receptor gene was encoded by eight exons. Since the leader peptide, transmembrane, and cytoplasmic regions are roughly encoded by exons 1, 7, and 8, respectively, each of these exons corresponds to a functional domain (Fig. 1). The exon and intron boundaries are located between the first and second letters of the codons except for the boundary between exons 7 and 8, which are split after the second letter. Second, the sequences of the coding regions of normal tissues were identical to the published cDNA sequence (12, 14), indicating that no point mutations had occurred in the protein-coding regions of the IL-2 receptor gene of the two ATL cell lines MT-1 and HUT102.

Homology of exons 2 and 4

Comparison of the amino acid sequences of different exons showed significant homology between exons 2 and 4, as shown in Fig. 4. The homologous residues were not highly clustered, but rather were dispersed throughout the exons. These results are most easily explained by the assumption that exons 2 and 4 were created by duplication of an ancestral exon. A similar region of homology was found out in the cDNA sequence by

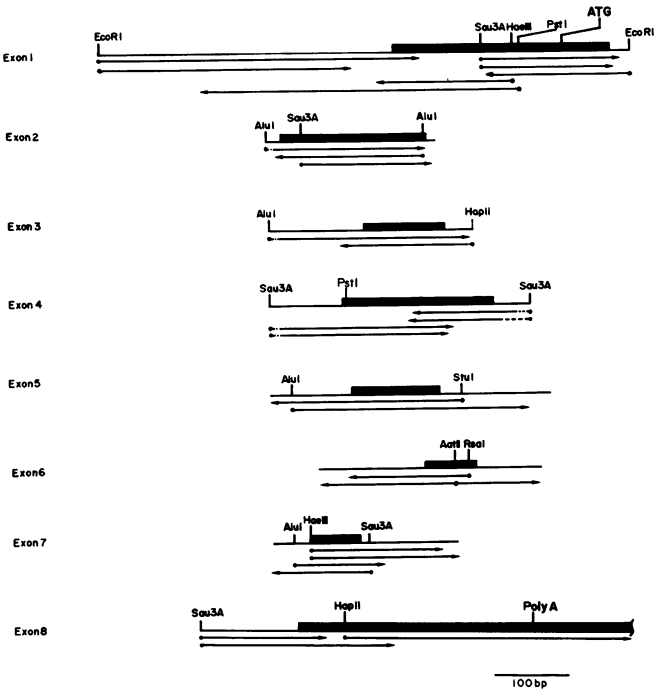

Fig. 2. Sequencing strategy of exons. Horizontal arrows indicate directions of sequences read. Broken lines show regions where sequences were not read. Vertical lines indicate restriction sites used for sequencing. Closed rectangles show exons. The initiation condon (ATG) and poly A addition signal are also indicated.

Cosman et al. (15). They suggested the possibility that exon 4 is generated by a transposable element, because the second homology region in the cDNA sequence corresponding to exon 4 is flanked by 8-bp direct repeats (Fig. 3). The 5' repeat was at the end of exon 3 and the 3' repeat at the end of exon 4. It is particularly interesting that the same sequence was found in the intron immediately 5' to exon 4. Thus, exon 4 was surrounded by direct repeats in the genomic sequence as well. However, the 8-bp repeat sequence is absent in the mouse IL-2 receptor cDNA (13), suggesting that the direct repeat sequences are not involved in the exon duplication. The deletion of the sequence corresponding to exon 4 in short mRNA (14, 15) could be due to misrecognition of the homologous sequences at the 3'-ends of exons 3 and 4 by the splicing machinery. The defective IL-2 receptor molecule produced by cDNA that deleted exon 4 in COS cells showed the loss of the reactivity with anti-Tac and 2A3 monoclonal antibodies and the

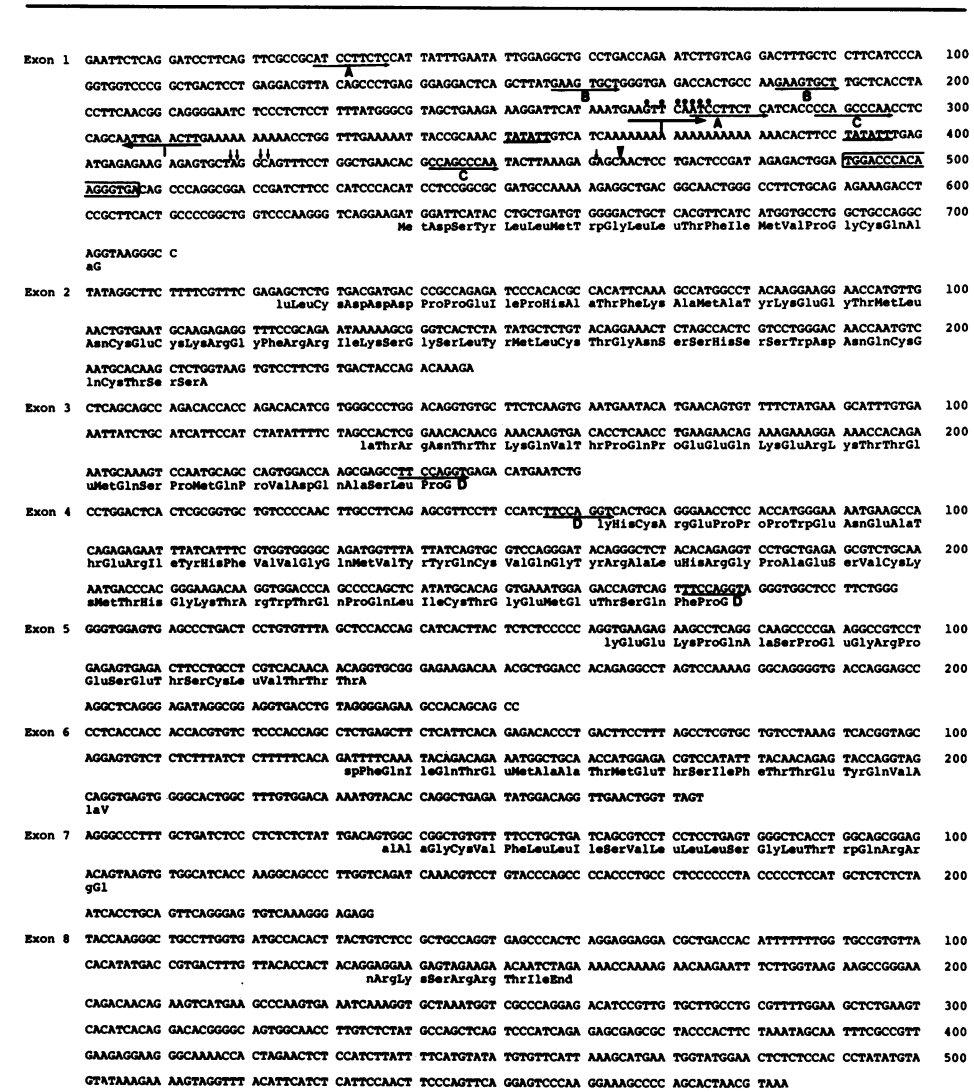

Fig. 3. Exon sequences of human IL-2 receptor gene. Exon I contains a promoter region and leader peptide. Horizontal arrows (A, B, C, and D) indicate direct repeats. Inverted repeats (I) are shown by thick horizontal arrows. Two TATA boxes are underlined. The sequence complementary to synthesized primer (17 bp) used in primer extension analysis is boxed. Vertical arrows indicate four sites of transcription initiation. The arrow head shows the 5'-end of cDNA clone Tac 2 (12). Closed circles above bases indicate CAAT-like sequence.

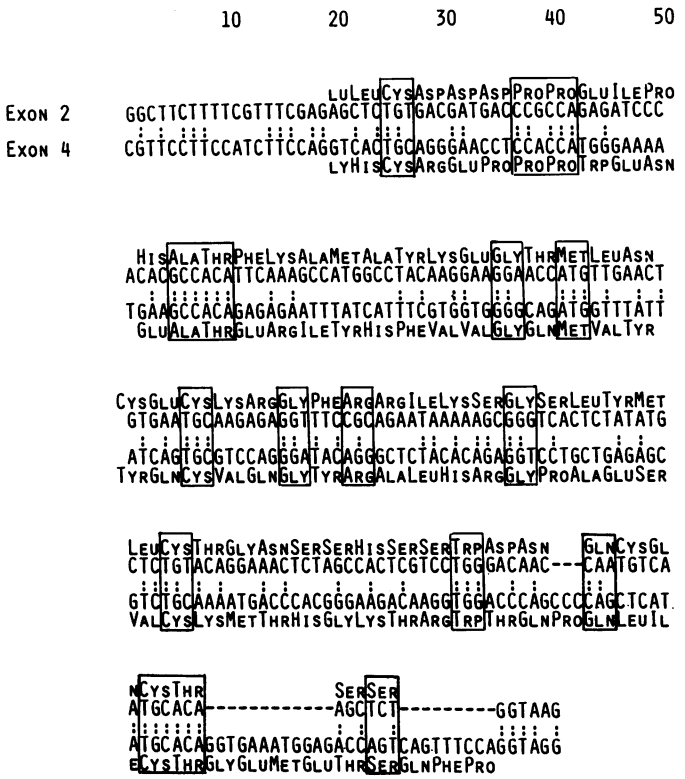

Fig. 4. Homology between exons 2 and 4. Upper and lower sequences are exons 2 and 4, respectively. Homologous amino acids are boxed. Vertical dots indicate homologous nucleotides. Deletions were introduced to maximize homology.

absence of the binding activity to IL-2 (14, 15). Since human IL-2 is effective in both human and murine T cells, the structure conserved between the two species may be functionally important. Comparison between mouse and human IL-2 receptor genes indicates that exon 2 (64.1%) and exon 4 (65.3%) are more conserved than other extra-cytoplasmic regions (13). These results suggest that the regions encoded by exons 2 and 4 of the IL-2 receptor gene may be important in IL-2 binding ability.

Pfeffer and Ullrich (22) found that receptors for plasma low-density lipoprotein (LDL) and epidermal growth factor (EGF), and EGF precursor (a receptor-type structure) contain a cysteine-rich repeated sequence. Eight repeated cysteine-rich regions may contribute to a looped, rigid

LDL-binding domain in the receptor (23). All 12 of the cysteine residues of the human IL-2 receptor are conserved in the murine receptor (13). Four out of six cysteine residues of exon 2 were conserved in exon 4 in both human and murine IL-2 receptors (Fig. 4). Exons 5 and 7 contained one cysteine residue each that is conserved between man and mouse. The extracellular region of the IL-2 receptor contained two cysteine-rich domains (encoded by exons 2 and 4) containing 60 - 72 amino acids, analogous to the other receptors mentioned above. Some of the cysteine residues conserved in exons 2 and 4 may form disulfide bonds between each pair if the domains encoded by these exons are oriented side by side in the same direction. Such a structure may have a pocket for binding IL-2.

Characterization of the promotor sequence

To identify the transcription initiation site, we did primer extension experiments using a synthetic 17-bp nucleotide complementary to the sequence 132-148 bp upstream of the initiation codon. The initiation sites were multiple, major sites being 29, 68, 69, 71, and 72 bp upstream of the 5'-end of the primer as shown in Fig. 5. The initiation site closest to the 3'-end, as identified by primer extension, was close to the 5'-end of the longer cDNA clone previously isolated (12) (Fig. 3). Although there were some variations in the relative intensity of the initiation sites, the locations of the sites were similar among all the mRNA of activated and HTLV-transformed T cell lines. We identified two TATA boxes within a 70-bp region upstream of the 5' initiation site (Fig. 3). The 3'-TATA box may be responsible for most of the initiation sites detected because the distance from the 5'-cluster of initiation sites was 23 - 27 bp, which is common to many eukaryotic genes. However, we cannot exclude the possibility that both TATA boxes are functional.

There were several interesting sequences in the 5'-flanking region of the IL-2 receptor gene (Fig. 3). Inverted repeat sequences (9-bp) with a 31-bp spacer were found 76-bp upstream of the 3'-TATA box. There were three pairs of direct repeats of 8 - 9-bp with spacers of various lengths. A CCAAT-like sequence (24) was located 115-bp upstream of the 3'-TATA box. There was a long A cluster (A_{21}) 8 bp 5' to the 3'-TATA box. A shorter A cluster (A_{10}) is located 26 bp 5' to the 5' TATA box. We do not know if these A clusters have any functional significance, although the clusters were also conserved in the murine IL-2 receptor gene (unpublished).

We compared the nucleotide sequences of the promotor regions of the genes encoding IL-2 (27) and its receptor because both genes are activated

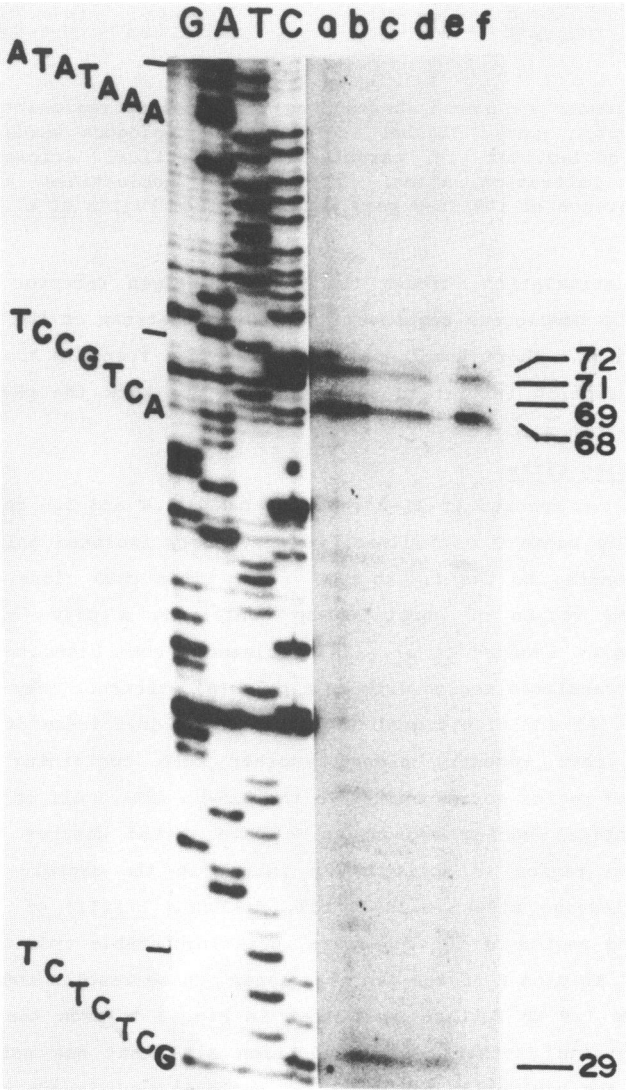

Fig. 5. Transcription initiation sites identified by primer extension. Lanes shown in the right indicate primer extension analysis. The sequence shown at the left was identified using the same primer except for the absence of the 5'-phosphate as used for primer extension with M13 phage clone containing the sense-strand. Numbers show nucleotides from the 5'-end of the primer. The extended primers run slightly faster than the corresponding bands in the sequencing gel because of one additional phosphate charge. Origins of RNA are: lane a, ATL-2; lane b, MT-1; lane c, YT; lane d, YT cells stimulated by IL-2 and ATL derived factor (26); lane e, normal T; lane f; normal T cells stimulated by IL-2.

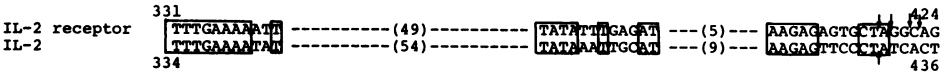

Fig. 6. Homologous sequences surrounding the promotor regions of the IL-2 and IL-2 receptor genes. Dashes indicate non-homologous sequences, with numbers of nucleotides in parentheses. Vertical arrows indicate transcription initiation sites. Homologous nucleotides are boxed. Nucleotide sequence of the IL-2 gene was taken from Fujita et al. (27).

upon antigen stimulation through the T cell antigen receptor (Fig. 6). There is a 9-bp homologous sequence about 50 bp upstream of the TATA boxes of the two genes. Short homologous sequences were found in the region 5' to the transcription initiation site. We do not know the physiological meaning of these homologies.

Poly (A) addition sites

Although two species of IL-2 receptor mRNA (1.4 and 3.5 kb) are present in various human T cell lines, we previously isolated only the cDNA clone corresponding to the 1.4-kb mRNA (12). This cDNA clone contains a 3'-untranslated region of about 320-bp containing a poly (A) addition signal (ATTAAA). Leonard et al. (14) isolated a cDNA clone containing a 1340-bp 3'-untranslated region without a poly (A) stretch. They identified a second poly (A) addition signal (AATAAA) 225-bp downstream of the first signal. We have recently cloned another cDNA containing a 2.3-kb 3'-untranslated region corresponding to the 3.5-kb mRNA. All of these cDNA have the identical coding regions. We have tested whether the 2.3-kb 3'-untranslated region is split by an intron in the genomic DNA. The restriction cleavage sites (with HincII, HpaI and HaeIII) of the 2.3-kb 3'-untranslated region of the cDNA were indistinguishable from those found immediately 3' to exon 8 of the genomic clone. These results indicate that exon 8 extends 1.9 kb further than shown in Fig. 3 towards the 3' direction, and contains another poly(A) addition site that has not yet been identified by the nucleotide sequence. S1 nuclease experiments showed that all of the three poly (A) addition signals were used in normal as well as transformed T cells (25).

ACKNOWLEDGEMENTS

We thank Mr. Y. Taniguchi and Dr. J. Yodoi for preparation of T cell mRNA. We are grateful to Ms. A. Kasahara and Y. Utsunomiya for expert technical assistance and to Ms. K. Fukui, S. Okamoto, and K. Hirano for preparation of the manuscript. This investigation was supported by grants

for the special promotion of science, and grants-in-aid from the Ministry of Education, Science, and Culture of Japan, from the Mochida Memorial Foundation for Medical and Pharmaceutical Research and from Yamanouchi Foundation for Research on Metabolic Disorders.

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