The Human IL-2 Receptor Gene Contains a Positive Regulatory Element That Functions in Cultured Cells and Cell-free Extracts*

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Noboru Suzuki, Norisada Matsunami, Hiroshi Kanamori, Norio Ishida, Akira Shimizu, Yoshio Yaota, Toshio Nikaido, and Tsukku Honjo

From the Department of Medical Chemistry and Department of Molecular and Cellular Biology, Kyoto University Faculty of Medicine, Sakyokukan, Kyoto 606, Japan

The 130-base pair fragment located between 220 and 90 base pairs upstream of the major transcription initiation site of the human interleukin 2 (IL-2) receptor gene had positive regulatory effect on the early promoter of simian virus 40 as well as its own promoter. This fragment seems to be responsible for not only cell-specific but also lymphokine-induced expression of the IL-2 receptor gene as assessed by DNA transfection. The same DNA fragment directed cell-specific transcription of the IL-2 receptor gene in extract of HTLV-I-infected T cells, MT-1, but not of Epstein-Barr virus-transformed B cells, CESS. The addition of small amounts of MT-1 extract to CESS extract resulted in specific expression of the IL-2 receptor gene, indicating that cell-specific expression is regulated by trans-acting molecules in MT-1 extract.

The physiological proliferation of T lymphocytes (T cells) requires specific interaction between a growth factor, interleukin 2 (IL-2), and its cell surface receptor (1). In normal T cells, expression of the IL-2 receptor is tightly regulated. IL-2 receptors are rapidly and temporally expressed on T cells only when they are stimulated with antigens, mitogens, or some other lymphokines. Normal resting T cells, which do not express the IL-2 receptor, fail to receive the growth signal. Regulated expression of the IL-2 receptor appears to provide the molecular basis of antigen-specific proliferation of T cells.

On the other hand, leukemic cells and cell lines derived from patients with adult T cell leukemia (ATL), which are infected with the human T cell leukemogenic retrovirus (HTLV-I), constitutively express a number of IL-2 receptors without any stimuli of antigens or mitogens (2, 3). The aberrant expression of the IL-2 receptor might be involved in some stages of leukemogenesis of retrovirus-infected T lymphocytes (4-6). Studies on the regulation of the IL-2 receptor gene expression are, therefore, important for elucidation of the mechanisms of not only the physiological clonal expansion of T cells but also leukemogenesis of T cells by HTLV-I.

For this purpose, we and others have cloned human and murine IL-2 receptor cDNAs (7-10) and then isolated the human genomic gene for the IL-2 receptor (11, 12). There were neither rearrangements of the IL-2 receptor gene nor alteration of the mRNA length in ATL-derived T cells. The nucleotide sequences of the coding regions of the human IL-2 receptor genes of the normal and the ATL-derived T cells were identical. Integration sites of the retrovirus in various ATL cells were not confined to a unique locus (13). The results suggested that the constitutive expression of the IL-2 receptor in ATL cells was ascribed to altered regulation of the IL-2 receptor synthesis rather than structural alteration of the IL-2 receptor gene per se and its surroundings.

We have compared the 5' flanking regions of the human and murine IL-2 receptor genes and found a relatively conserved region within the 250-base pair (bp) region 5' to the transcription initiation sites. The conserved region exerted a positive regulatory effect on expression of the IL-2 receptor gene in IL-2 receptor-expressing T cells as well as in cell-free extracts derived from IL-2 receptor-expressing cells. We have found that the enhancer-like regulatory element located in the conserved region and trans-acting molecule(s) are responsible for cell-specific expression of the IL-2 receptor and its induction by a lymphokine.

MATERIALS AND METHODS

Cells—Epstein-Barr virus-transformed human B cell line CESS (14), HTLV-I-infected human leukemic T cell lines MT-1 and MOLT-4 (15, 16), human leukemic T cell line MOLT-4, and human NK-like T cell line YT-C9 (17) were grown in RPMI 1640 medium containing 10% fetal calf serum. HeLa cells for transfection were grown on a dish in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. HeLa cells for cell extracts were grown in suspension culture in Eagle's minimal essential medium containing 10% fetal calf serum.

Cloning of Recombinant Phages—Phages were screened by the method of Benton and Davis (18). Isolation of the phage designated 5-9 which contained a part of the intron 1 of the human IL-2 receptor gene was carried out as described elsewhere (11). The 5' flanking region of the murine IL-2 receptor gene was cloned from the Charon 4A phage library containing EcoRI partial digests of C57BL DNA (a gift of J. Seidman of Harvard University) using as probe the 0.4-kb PstI-PstI fragment of the mouse IL-2 receptor cDNA (8). The clone

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2 Present address: Cell Science and Technology Division, Fermentation Research Institute, Ministry of International Trade and Industry, Ibaragi 305, Japan.

3 Present address: Department of Genetics, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115.

4 Present address: Laboratory of Molecular Biology, Aichi Cancer Center Research Institute, Nagoya 464, Japan.

5 The abbreviations used are IL-2, interleukin 2; HTLV-I, human T cell leukemogenic retrovirus; ATL, adult T cell leukemia; ADF, ATL-derived factor; bp, base pair; DMEM, Dulbecco's modified Eagle's medium; kb, kilobase; Pipes, 1,4-piperazinediethanesulfonic acid; CAT gene, chloramphenicol acetyltransferase gene; LTR, long terminal repeat.

6 The sequences of the human IL-2 receptor gene in MT-1 and MT-2 (15, 16), human leukemic T cell line MOLT-4, and human NK-like T cell line YT-C9 (17) were grown in RPMI 1640 medium containing 10% fetal calf serum. HeLa cells for transfection were grown on a dish in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. HeLa cells for cell extracts were grown in suspension culture in Eagle's minimal essential medium containing 10% fetal calf serum.

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designated Ch. M. IL-2R9 contained the 16-kb insert of the 5' flanking region, exon 1, and a part of intron 1 of the murine IL-2 receptor gene. The nucleotide sequence of the region homologous to the 0.6-kb EcoRI-PstI fragment of the human IL-2 receptor gene was determined.

Construction of Plasmids—The EcoRI-PstI fragment (−419 +167) of the human IL-2 receptor gene was ligated with HindIII linker and inserted into the HindIII site of pSVOCAT. The plasmid pSVOCAT(−419) was cleaved by NdeI and subjected to Bal31 digestion for different intervals (Fig. 3A). The Bal31-digested plasmids were ligated with the NdeI linker and then cleaved with HindIII. NdeI-HindIII fragments of various lengths were isolated by electrophoresis and ligated with pSVOCAT which had been cleaved with NdeI and HindIII. Plasmids were grown, and deleted regions were determined by nucleotide sequence determination. The SmaI-RsaI fragment (U/R) of HTLV-I LTR (19) was inserted at the HindIII site of pSVOCAT for different intervals (Fig. 3A). The Bal31-digested plasmids were ligated with pSVOCAT(U/R), and the plasmid was designated as pSVOCAT(U/R).

Construction of pM5G2, pAd2 SmaI-F, and pVH101 was as described (20).

Nucleotide Sequencing—Cloned phage DNAs were digested by restriction enzymes. DNA fragments were subcloned at polylinker sites of M13 phage vectors (mp18, mp19, and mp18) or pUC vectors (vectors 18 and 19) and sequenced by the dyeoxy chain termination method (21).

DNA Transfection and Chloramphenicol Acetyltransferase Assay—Lymphocytes were transfected by the DEAE-dextran method as described by Queen and Baltimore (22) with slight modification. Culture media were all refreshed 16 h before transfection. Cells were harvested and washed twice with serum-free DMEM. Cells (5 × 10^6) were suspended in 0.75 ml of serum-free DMEM, and then 1:1 mixtures of DEAE-dextran and plasmid DNA dissolved in serum-free medium were added. The cells were incubated for 20 min at 37°C and then heparin was added to about 5 units/ml. Subsequently, the cells were washed twice with serum-free DMEM and incubated in the media described above. Optimal concentrations (final) of DEAE-dextran were 50, 200, 200, 250, and 800 μg/ml for CESS, MT-1, MT-2, MOLT-4, and YT-C3, respectively. HeLa cells were transfected using calcium phosphate precipitation (23). Two days after transfection, the cell culture supernatant was harvested and the chloramphenicol acetyltransferase activity of cell extracts was measured as described by Gorman et al. (24).

pSV2CAT was always included as positive control. ADF used as the culture supernatant of HTLV-I-infected T cell line, ATL-2 (6). ADF was added to the medium of YT-C3 to 2% immediately after transfection.

RNase Mapping—RNA products were characterized by RNase mapping using RNA probe made by the SP6 in vitro transcription system according to Melton et al. (25) except that hybridization was performed at 55°C overnight in 65% formamide, 40 mM Pipes (pH 6.7), 0.4 M NaCl, 1 mM EDTA. Total cellular RNA was isolated as described (26).

In Vitro Transcription Analysis—Preparation of whole cell transcription extracts from each cell line and in vitro transcription in these extracts were carried out according to the procedure of Manley et al. (27). Detailed protocol was described previously (20). RNAs were extracted with phenol and chloroform/isoamyl alcohol (24:1) and the extracts were precipitated by ethanol. RNAs were electrophoresed in 5% polyacrylamide, 7 M urea gel at 50 V/cm and the dried gels were autoradiographed. Adenovirus 2 (pA2 Sma1-F) plasmid was digested with SmaI to yield two separate fragments of the insert and vector, and their mixtures were used as a template. The mouse globin (pMG92) and the mouse immunoglobulin VH101 (pVH101) plasmids were linearized by digestion with BamHI and HindIII, respectively, and used as templates (20). Sizes of all the template DNA fragments were about the same.

RESULTS

Comparison of the 5' Flanking Sequences of the Human and Mouse IL-2 Receptor Genes—We have determined the nucleotide sequence of the 650-bp region upstream of the human IL-2 receptor gene, and identified two typical TATA boxes (positions −68−62 and −28−22) and two less conserved TATA-like heptanucleotides (positions +16+22 and +31+37; Ref. 28) as shown in Fig. 1. A part of this sequence was previously reported (11, 12). By RNase mapping, we have determined four transcription initiation sites at base positions −34 (C1), +1 (C2), +44 (C3), and +63 (C4), each being located 28−34 bp 3' to the first nucleotide of TATA-like sequences (Fig. 2). Although both C1 and C2 sites were preceded by the typical TATA boxes, transcripts initiated at C1 were much more abundant than those from C2. Transcripts from C4 were so little that we had been unable to detect them by the primer extension analysis (11). Only the most 3’ of the three CAAT box-like sequences found in the 5’ flanking region is located 70−90 bp upstream of the mRNA start site (C2), which is the common location of the functional CAAT box (28).

To identify further the sequences important for regulation of the IL-2 receptor gene, we isolated murine genomic clones of the IL-2 receptor gene, determined the nucleotide sequence of its 5’ flanking region, and compared it with the human sequence (Fig. 1). The most significant homology was found in the region immediately 5’ to the C2 site (from −280 to +60). It is striking and unique in mammalian promoters that poly(A) stretches, which are reasonably conserved between man and mouse, precede the two TATA boxes.

Cell-specific Expression Directed by the 5' Flanking Region of the Human IL-2 Receptor Gene—To test the regulatory activity of the 5' flanking DNA of the human IL-2 receptor gene, we have isolated the 886-bp EcoRI-PstI fragment (−419 to +167) in Fig. 1) containing the highly conserved region and inserted the fragment at the HindIII site located 5’ to the bacterial chloramphenicol acetyltransferase (CAT) gene of pSVOCAT (25). The plasmid was described as pSVOCAT(−419) as shown in Fig. 3. Similar plasmids containing two reference promoters, HTLV-I LTR(U/R) and the SV40 early promoter were also used. Cells were transfected with plasmid DNA, and cellular extracts were assayed for the chloramphenicol acetyltransferase activity as a measure of the promoter activity. Since efficiency of DNA transfection varied between cells, the promoter activities of the IL-2 receptor gene and HTLV-I LTR were normalized by the activity of the SV40 promoter.

As shown in Table I, pSVOCAT(−419) expressed the chloramphenicol acetyltransferase activity in MT-1 and MT-2 cells which contained the HTLV-I genome and constitutively expressed a number of IL-2 receptors. In MT-1 cells the IL-2 receptor promoter fragments inserted in front of the CAT gene directed transcription starting at the same four sites as identified for the endogenous MT-1 mRNA, although the relative intensity of each band was conserved that of the endogenous mRNA (Fig. 2). The results suggest that our transfection assay, if not completely, reflects in vivo transcription of the IL-2 receptor gene in MT-1 cells. By contrast, the IL-2 receptor promoter activity was not detectable in MOLT-4, CESS, and HeLa cells which did not express IL-2 receptors on their surfaces. The HTLV-I promoter showed the strong activity in HTLV-I-infected cells, MT-1 and MT-2, as reported before (29, 30). The results clearly indicate that the EcoRI-PstI fragment of the 5' flanking region of the IL-2 receptor gene contains the sequence that controls the cell-specific expression of this gene. HTLV-I-infected cells MT-1 and MT-2, in turn, seem to contain trans-acting molecules that regulate cell-specific expression of the IL-2 receptor gene as well as HTLV-I LTR.

Dissection of Regulatory Elements of the IL-2 Receptor Gene by Deletion Mapping—A series of deletions were introduced at the 5’ end of the EcoRI-PstI fragment (−419+167) by Bal31 exonuclease digestion as shown in Fig. 5B. The deleted fragments were inserted at the NdeI site of pSVOCAT(ΔH which deleted the 51-bp NdeI-HindIII fragment (pBR322 sequence) from pSVOCAT (Fig. 5A). The 51-bp deletion from the vector did not affect the assay system, as the background
Enhancer-like Element of IL-2 Receptor Gene

FIG. 1. Nucleotide sequences of the 5' flanking regions of the human and murine IL-2 receptor genes. Hyphens show deletions. Murine nucleotides different from the human sequence are shown. Insertions in the murine sequence are shown in parentheses below the preceding murine bases. Dots in the murine sequence indicate undetermined bases. TATA and TATA-like sequences are shown by triangles (position -135) and (position -124). Corrections of the previous sequence are shown by underlined. The ends of the deleted fragments shown in Fig. 3B are shown by vertical arrows. CAAT-like sequences are underlined. Corrections of the previous sequence (11) are insertions of two bases; A (position -135) and C (position -124).

activities of pSVOCAT and pSVOCATΔH were the same (data not shown). The expression of the chloramphenicol acetyltransferase activity in MT-1 cells was tested for each plasmid. The chloramphenicol acetyltransferase activity was not reduced drastically even when the deletion reached -221 as shown in Fig. 4A. However, the chloramphenicol acetyltransferase activity was greatly reduced when the deletion reached -180, and almost no activity was found when the deletion reached -127. The DNA sequence responsible for cell-specific expression of the IL-2 receptor gene seems to be located between -311 and -127.

5' Flanking Sequence Responsible for Induction by a Lympohokine—Expression of the IL-2 receptor is regulated by several lymphokines including IL-1 and ADF (17, 31). ADF is secreted by ATL cell lines and induces expression of the IL-2 receptor on a NK-like human T cell line YT-C3 cells. We have tested whether the same series of the deletion mutants of the 5' flanking region of the IL-2 receptor gene as described above respond to ADF in YT-C3 cells. Immediately after transfection with an equimolar amount of each plasmid, cells were equally divided into two dishes. One received ADF and the other did not. The chloramphenicol acetyltransferase activity expressed by each recombinant DNA was measured 48 h after transfection. As shown in Fig. 4B, ADF induced expression of the chloramphenicol acetyltransferase activity using pSVOCAT(-419) as template. The response to ADF was markedly reduced when the deletion of the 5' flanking region passed -221. The profile of ADF-dependent induction of the chloramphenicol acetyltransferase activities of various deletion mutants in YT-C3 cells was similar to that of the chloramphenicol acetyltransferase activities expressed by the mutants in MT-1 cells.

Enhancer-like Element in the 5' Flanking Region of the IL-2 Receptor Gene—We then tested whether the upstream segment of the IL-2 receptor gene had any enhancer-like element. The 586-bp EcoRI-PstI fragment (-419/+167) was inserted at the BamHI site located 3' to the CAT gene of an enhancer test vector pAloCAT, in which the CAT gene was preceded by the SV40 early promoter without its enhancer (Fig. 3A). As shown in Table II, the insertion of the EcoRI-PstI fragment gave rise to 3-4-fold enhancement of the chloramphenicol acetyltransferase activity as compared with the original pAloCAT, in MT-1 cells (Experiments 1-1 and 1-2) but not in CESS cells (Experiments 3-1 and 1-2) regardless of the orientation of the inserted DNA. The results indicate that the EcoRI-PstI fragment has the cell-specific enhancer-like activity.
We have also tested some of the deleted segments of the EcoRI-PstI fragment for the enhancing activity of the SV40 promoter. Again, the enhancing activity was reduced when deletion from the 5' end passed -221 and completely abolished when deletion extended to -91. The DNA fragment between -221 and -91 was isolated and inserted at the BamHI or BglII site of pA1,CAT (Experiment 1). The -221/-91 DNA segment showed a 10-fold more stimulation to the IL-2 receptor promoter (Experiment 2-2 and 2-3). pSVOCATAH(-221) is considered as a derivative of pSVOCATAH(-91) which had the -221/-91 fragment inserted at the NdeI site in the normal orientation. pSVOCATAH(-221) showed 10 times higher chloramphenicol acetyltransferase activity than that containing the same fragment at BamHI site (Experiment 2-4). The difference might be due to the distance from the promoter rather than location relative to the promoter; the distances from the major start site to BamHI and NdeI sites are 1.7 and 0.1 kb, respectively. The -221/-91 DNA segment showed 10-fold more stimulation to the IL-2 receptor promoter (Experiment

![Fig. 2. Determination of the transcription start sites of the IL-2 receptor promoter in MT-1 cells.](image)

![Fig. 3. Structures of vectors and deleted fragments for promoter and enhancer assays.](image)

| Host cell lines | CAT activities directed by promoters of | IL-2R LTR SV40 |
|-----------------|----------------------------------------|---------------|
| MT-1 T cells    | HTLV-1 +                             | 22 165 100    |
| MT-2 T cells    | HTLV-1 +                             | 45 1376 100   |
| MOLT-4 T cells  | None -                               | 0 0 100       |
| CESS B cells    | EBV -                                 | 0 0 100       |
| HeLa Epithelial cells | None -                  | 0 5 100       |
point of deleted promoter fragment and plotted on the abscissa. The exons 1 and well. We conclude that the -221/-127 segment has the en-

pSVOCATAH vector. The mutants were represented by the promoter activity in MT-1 cells probably because of the gene (32).

position is as described in the legend to Fig. 1. Background activity of pSVOCAT was less than 2%. B, deletion mutants were tested for the promoter activity in the presence (induced) or absence (control) of ADF in YT-C3 cell. Mutants are plotted as in A. Experiments were repeated more than three times and the profile was essentially identi-
cap, initiation site (C).

2-4) than to the SV40 promoter (Experiment 1-9). A similar synergistic effect between the enhancer element and its original promoter was recently described for the immunoglobulin gene (32).

Deletion of the -221/-127 segment from the 5' flanking region of the IL-2 receptor gene gave rise to the reduction of the promoter activity in MT-1 cells probably because of the loss of the enhancer-like element. Since the loss of the same DNA segment also abolished the induced expression in re-

sponse to ADF in YT-C3 cells, the enhancer-like element seems to be responsible for induction by the lymphokine as well. We conclude that the -221/-127 segment has the enhancer-like activity and plays an important role in regulation of the IL-2 receptor gene expression.

We also tested whether another regulatory element was located anywhere in the 38-kb intervening sequence between exons 1 and 2. The whole intron between exons 1 and 2 was cloned in four phage clones (Fig. 5). The clone designated 5-9 linked the previously isolated clones b6 and B26 (11). These clones were digested with EcoRI, and resultant fragments were inserted into the BglII site of pA2CAT. No enhancing activity was detected in any fragments derived from the first intron of the human IL-2 receptor gene. We do not exclude the possibility that other control elements might be detected by other assay systems.

**Preferential Transcription of the IL-2 Receptor Promoter in Cell-free Extract of MT-1 Cells**—To study the regulatory mechanism of the IL-2 receptor gene further, it is important to establish a cell-free system that allows accurate and regu-

lated expression of the promoter function. Such a system will facilitate purification and characterization of trans-acting molecules which might be involved in regulatory expression of the IL-2 receptor gene. For the in vitro transcription reaction we constructed a template plasmid of the IL-2 recep-
tor gene, pUC·H·IL-2R(E-E) that contains the 728-bp EcoRI fragment encompassing the 5' flanking region (-419/+220), exon 1, and a portion of intron 1 as shown in Fig. 6. We used HindIII-cleaved pUC·H·IL-2R(E-E) as a truncated template which was expected to give rise to a 356-base runoff product upon the faithful initiation of transcription from the C2 start site (Fig. 1). To study the promoter specificity of transcription reactions, we also used template plasmids containing three other well-characterized class II genes (20): the major late gene of adenovirus 2 (Ad2ML) (35), the mouse β-globin gene (34, 35), and the mouse immunoglobulin VH101 gene (36),

**Table II**

Enhancer-like element in the 5' flanking region of the IL-2 receptor gene

Segments of the 5' flanking region of the human IL-2 receptor gene, which are indicated by the positions of both ends (Fig. 1), were inserted into the BamHI and BglII sites of pA2pCAT which were located upstream and downstream of the CAT gene, respectively (Experiments 1 and 3). Plasmids were linearized by cleaving at the PstI site in the Amp' gene before transfection to avoid read-through transcription from the downstream promoter via the vector DNA. The -221/-91 fragment was inserted at the BamHI and the NdeI sites of pSVOCATΔH(-91) which are located downstream and upstream of the CAT gene, respectively (Experiment 2). In Experiment 2-4, pSVOCATΔH(-221) was used. Chloramphenicol acetyltransferase (CAT) activities of cell extracts were measured after introduction of each plasmid into MT-1 cells (Experiments 1 and 2) or CESS cells (Experiment 3). The chloramphenicol acetyltransferase activity is shown by ratio to that obtained using the parental plasmid pA2pCAT in Experiments 1 and 3 or pSVOCATΔH(-91) in Experiment 2. Radioactivities (counts/minute) of acetylated chloramphenicol measured were normalized and are shown in parentheses. The total radioactivities (counts/minute) of chloramphenicol recovered in Experiments 1, 2, and 3 were 344,400, 425,400, and 325,900, respectively.

| Experiment | Inserted DNA | Segments | Locations relative to promoter | Orientations | CAT activity |
|------------|--------------|----------|---------------------------------|--------------|--------------|
| 1          | -221/-127    | None     | Downstream                      | Normal       | 1 (769)      |
| 2          | -221/-91     | None     | Downstream                      | Normal       | 1 (3,579)    |
| 3          | -221/-60     | None     | Downstream                      | Normal       | 1 (1,225)    |
| 4          | -221/-60     | None     | Downstream                      | Reverse      | 0.5 (858)    |
| 5          | -221/-30     | None     | Downstream                      | Normal       | 0.5 (858)    |
| 6          | -221/-30     | None     | Downstream                      | Reverse      | 0.5 (858)    |
| 7          | -221/-30     | None     | Downstream                      | Normal       | 0.5 (858)    |
| 8          | -221/-30     | None     | Downstream                      | Reverse      | 0.5 (858)    |
| 9          | -221/-30     | None     | Downstream                      | Normal       | 0.5 (858)    |
| 10         | -221/-30     | None     | Downstream                      | Reverse      | 0.5 (858)    |

**Fig. 5. Restriction map of the first intron of the human IL-2 receptor gene**—Exons are indicated by boxes numbered from the 5' end of transcription. EcoRI sites are indicated by arrows. Heads. Phage clones spanning the first intron of the human IL-2 receptor gene are shown below: 5-9 is newly isolated and other clones were previously reported (11).
The Enhancer-like Element of the IL-2 Receptor Gene Is Essential for Preferential Transcription in MT-1 Extract—

Some of the deletion mutants of the 5' flanking region of the IL-2 receptor gene were tested in a cell-free transcription system of MT-1 cells. We used pSVOCAT(-419), pSVOCATΔH(-311), pSVOCATΔH(-221), pSVOCATΔH(-127), and pSVOCATΔH(-34) (Fig. 2B). All of these plasmids were cleaved at the PvuII site located in the CAT gene and used as templates, which should yield 320-base runoff transcripts. The plasmid templates produced 320-base transcripts until deletions exceeded -221 as shown in Fig. 8. The plasmids containing deletions beyond -221 markedly diminished the production of 320-base RNA. The promoter activity profile of deletion mutants in the MT-1 cell-free extract was similar to that obtained by DNA transfection assay in MT-1 cells (Fig. 4). The results indicate that the enhancer-like element located between -221 and -127 is involved in cell-specific expression of the IL-2 receptor promoter in the MT-1 cell extract.

The 320-base run-off product corresponded to RNA transcribed from the most prominent start site (C2, position +1).
Although the high background on the gel hindered the detection of the runoff products initiated at the other three sites (C5, C6, and C4), we identified all the four runoff products initiated from the C5, C6, C7, and C7, start sites by RNAS mapping in the experiment using pSVOCATΔH(-311) and the MT-1 extract (data not shown). The background is higher than the experiments described in the legend to Fig. 7 probably because of the longer templates.

**Trans-acting Molecules in MT-1 Cell Extract Stimulate Transcription of the IL-2 Receptor Promoter in CESS Cell Extract**—We have tested whether low transcription efficiency of the IL-2 receptor promoter in the CESS extract is due to the absence of trans-acting molecules that regulate expression of the IL-2 receptor gene. pUC-H-IL-2R(E-E) was transcribed in mixtures of CESS and MT-1 extracts with different ratios. The addition of small amounts of the MT-1 extract to the CESS extract strongly enhanced synthesis of the 355-base runoff product of the IL-2 receptor promoter as shown in Fig. 9. The control experiment showed that the transcription of the VH101 promoter remained constant in various mixtures. Transcriptional activities were quantitated by densitometer tracing of the runoff products. Experimental results were compared with values calculated by mixed proportions of the two extracts. As summarized in Table III, the addition of the MT-1 extract to the CESS extract enhanced transcription of the IL-2 receptor promoter to the extents that exceeded the calculated values, indicating that trans-acting molecules in MT-1 were able to cooperate with the transcription system in CESS. In other words, the trans-acting molecules which regulate the expression of the IL-2 receptor gene are limiting in the CESS cell extract.

**DISCUSSION**

**A Cell-specific Regulatory Region That Exerts Positive Transcriptional Control**—We have identified a positive regulatory element at the −220/−90 region of the human IL-2 receptor gene. Removal of this region abolished the transcriptional capacity of the IL-2 receptor promoter that directed preferential expression of the CAT gene in the human T cells expressing the IL-2 receptor. The same element seems to be responsible for positive regulation of the IL-2 receptor gene upon stimulation by the ADF lymphokine. We showed that this sequence had an enhancer-like activity. Since the profiles of transcription activities of the deletion mutants in both MT-1 cells and the ADF-induced YT-C3 cells are essentially similar, it is likely that the manner of the activation of the IL-2 receptor gene is common in both cell lines. As the simplest interpretation, we propose that the upstream control element interacts with trans-acting molecules. Trans-acting molecules which interact with the enhancer-like region might be induced in the activated YT-C3 cells.

**Possible Involvement of Trans-acting Molecules**—It is a clue to reconstitute a cell-free transcription system for elucidation and isolation of transcription control factor(s). We demonstrated the cell-specific expression of the human IL-2 receptor promoter in cell-free transcription systems. The positive regulatory element was shown to be responsible for cell-specific transcription of the IL-2 receptor gene in the MT-1 cell extract. Removal of this DNA segment abolished synthesis of the runoff transcripts initiated by the IL-2 receptor promoter. Addition of small amounts of the MT-1 extract to the CESS cell extract showed a marked stimulation of expression of the IL-2 receptor promoter, indicating the presence of trans-acting molecule(s) in MT-1 cells. It is likely that the trans-acting molecules interact with the enhancer-like element of the IL-2 receptor gene.

The close association of the aberrant expression of IL-2 receptors with the infection of human C-type retrovirus HTLV-1 suggested the possibility that the IL-2 receptor gene is directly or indirectly activated by viral product(s). It has been reported that the p40* (tat) protein encoded by the pX region of HTLV-I activates the promoter of the viral genome (37, 38). Greene et al. (39) showed that transfection and expression of the pX gene induced expression of the IL-2 receptor in Jurkat cells. However, it is not clear whether or not the activation of the IL-2 receptor gene is caused by direct interaction of p40* protein with the regulatory element of the IL-2 receptor gene. Our cell-free system will provide a means to test a direct effect of the p40* protein when the purified material is available.

**Suppression by Further Upstream Element of the 5' Flanking Region of the IL-2 Receptor Gene**—The chloramphenicol acetyltransferase activity of pSVOCAT(−419) was lower than that of pSVOCATΔH(−311). pSVOCAT plasmids containing the upstream fragment beyond −419 were less active than pSVOCAT(−311). Plasmids containing a further upstream region (−3000 to −2000) of the human IL-2 receptor gene showed a substantially reduced transcriptional activity. Since the insertion of the −2000/−419 or −3000/−2000 segment upstream or downstream of the SV40 early promoter of pSV2CAT did not reduce the chloramphenicol acetyltransferase activity.

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*Y. Yaoita, T. Nikaido, and T. Honjo, unpublished data.*
ase activity (data not shown), the upstream fragment of the IL-2 receptor gene seems to differ from a negative regulatory element of repetitive sequences which was found upstream of the enhancer element of the rat insulin 1 gene (40).

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