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# Identification of multiple *cis*-elements and *trans*-acting factors involved in the induced expression of human IL-2 gene

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## ABSTRACT

Regulated expression of interleukin-2 (IL-2) gene constitutes an essential part in the clonal proliferation of activated T lymphocytes (T-cells). In order to gain insight on the mechanism(s) of the IL-2 gene induction, deletions were introduced in the human IL-2 gene 5'-flanking region and the mitogen inducibility of each deletion mutant was examined in cultured T cell lines. At least four functional regions were identified, they contain potential binding sites for several transcription factors including one NF-$\varkappa$B and two octamer binding factor sites. Whereas each of the functional regions (or elements) is required for the maximal induction of the IL-2 gene by mitogen, one such region was found to be dispensable for activation by the HTLV-1-encoded *trans*-activator, *tax*-1. Furthermore, the potent immunosuppressive agent, cyclosporin A was found to inhibit the gene induction by mitogen, but not by *tax*-1.

## INTRODUCTION

Interleukin-2 (IL-2) is a lymphokine produced exclusively by activated T lymphocytes (T-cells). The biological effects of IL-2 is exerted by the interaction of IL-2 with a specific high-affinity receptor complex which consists of two IL-2 binding components; IL-2 receptor $\alpha$ chain (IL-2R$\alpha$, p55) and IL-2 receptor $\beta$ chain (IL-2R$\beta$, p70−75) (1). Expression of the genes encoding IL-2, IL-2R$\alpha$ and IL-2R$\beta$ is induced in activated T-cells (2−4). In fact, constitutive mRNA expression is observed for IL-2R$\beta$ gene albeit at low levels, but not at all for IL-2 and IL-2R$\alpha$ genes in peripheral blood lymphocytes unless stimulated by mitogens (4). Since IL-2 is a major lymphokine for T cell proliferation, the regulated expression of these genes constitutes an essential part in the control mechanism of T-cell clonal expansion. Indeed, evidence for the involvement of an aberrant expression of IL-2 and/or IL-2R genes in T cell transformation has been provided, in particular, for Adult T cell leukemia (ATL) which is caused by a retrovirus, the human T cell leukemia virus type I (HTLV-1) (5, 6).

Recently, we and others have identified DNA sequences in the 5'-flanking region which were found to be responsible for the regulated expression of human IL-2 and IL-2R$\alpha$ genes in the human T cell line, Jurkat (6−9). Similar observations have also been reported for the mouse IL-2 gene (10). We have previously demonstrated by using a co-transfection assay that *tax*-1 encoded by HTLV-1, transcriptionally activates both IL-2 and IL-2R$\alpha$ genes in the absence of mitogenic stimulus (6, 11). Moreover, recent studies report the identification of several nuclear factors which bind to the IL-2 and IL-2R$\alpha$ gene sequences (10, 12−15).

To gain further insight on the mechanism(s) of IL-2 gene induction by mitogen or *tax*-1 in T-cells, we attempted to dissect further the *cis*-acting regulatory DNA sequences which

 

are required for the human IL-2 gene activation. In this study, we demonstrate that the 5′-flanking sequence of the IL-2 gene can be tentatively divided into at least four functional regions which together mediate the mitogen-induced expression of the gene at maximal levels. We provided evidence that one of these regions is not required for maximum activation by *tax*-1. We also demonstrate that at least four factors bind to these functional regions. Furthermore, we present evidence that cyclosporin A (CsA), a potent immunosuppressive agent, inhibits mitogen-induced, but not *tax*-1-mediated activation of the IL-2 gene.

## MATERIALS AND METHODS

*Construction of Plasmids*

The plasmids pSI319cat, pSI264cat, pSI222cat, pSI151cat and pSI138cat were constructed essentially as described previously (15). In order to prepare the 3′-deletion mutants, pIL2−319cat was first cleaved by HindIII and treated by Bal31, then the resulting DNA termini were ligated with BamHI linker. Subsequently, the Bal31-treated DNA, containing the 5′-flanking sequence was excised by digestion with XbaI and cloned into the XbaI- and BamHI-digested pSIFN-β66cat in which the interferon-β (IFN-β) 'silent' promoter (−66 to +19) (16) is inserted into the HindIII site of pSV0cat (17). The IFN-β promoter contains one NF-κB motif GGGAAATTCC (18). In this procedure, pSI319-33cat, pSI319-60cat, pSI319-98cat, pSI319-115cat, pSI319-127cat and pSI319-145cat were obtained. The deletion endpoint of each mutant was determined by sequencing.

*DNA Transfection, CAT, gpt Activity Assay and S1 Mapping Analysis*

The DNA transfection, mitogen stimulation, CAT and *gpt* assays, RNA preparation and S1 analysis were carried out essentially as described previously (15, 19). The mutant gene as described above was transfected into the human T-cell line, Jurkat, or the mouse T-cell line, EL-4, using pRSVgpt (19) or pRSVtk (15) as the internal reference genes for CAT assay and S1 mapping analysis, respectively. In determining the relative inducibilities by CAT assay and S1 analysis, the induced CAT activities and mRNA levels were normalized by the *gpt* activity and the *tk* mRNA levels respectively in each sample. The CAT gene expression was monitored after mitogen stimulation of the transfected cells either by ConA (25 μg/ml; Jurkat) or by TPA (10 ng/ml; EL-4) as described previously (7). To examine the effect of CsA (Sandoz Pharmaceutical), the compound was added simultaneously with ConA to the Jurkat cell cultures at a final concentration of 1 μg/ml.

*Nuclear Extract Preparation and Gel-retardation Assay*

Nuclear extract preparation and gel-retardation assays were performed as described previously (15). The $^{32}$P-labeled DNA probes (phosphorylated at the 5′-terminus of both strands) were prepared by isolating a 37 bp XbaI-DdeI fragment from pIL2-264cat, a 58 bp XbaI-DraI fragment from pIL2-222cat, a 50 bp DraI-BamHI fragment from pSI319-115cat and a 56 bp XbaI-BamHI fragment from pSI319-107cat which encompass the regions from −228 to −264, from −165 to −222, from −115 to −164 and from −52 to −107 with respect to the CAP site, respectively. Essentially, DNA probe for immunoglobulin (Ig) octamer motif was prepared by synthesizing the following sequences (20):

5′-TATTTTAGAAATGCAAATTACCCAGGTGGTC-3′
3′-ATAAAATCTTTACGTTTAATGGGTCCACCAG-5′
octamer motif

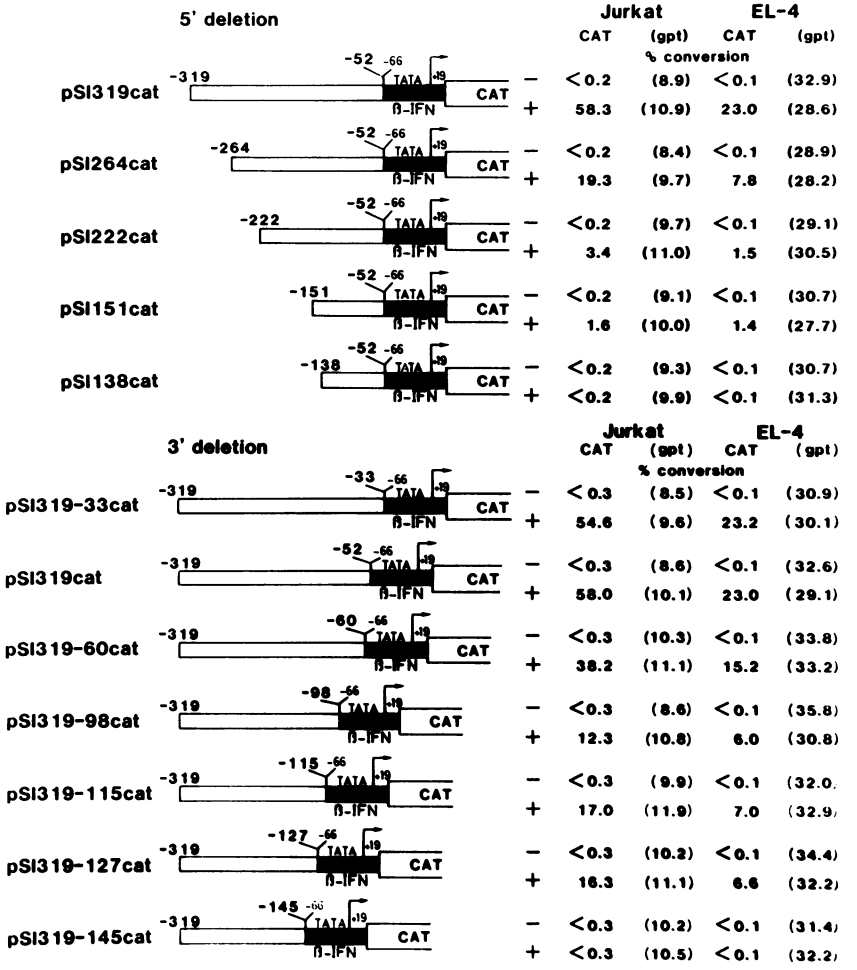


**Figure 1.** Expression of the various IL-2 deletion mutants in human and mouse T-cell lines. The structure of the various IL-2 deletion mutants are illustrated above. Open blocks represent the 5'-flanking DNA sequences of the IL-2 gene. TATA represents the consensus TATA box. The transcription initiation site of the IFN-$\beta$ gene is indicated by an arrow. The Jurkat cells transfected with these gene constructs were either treated with ConA (+) or mock-treated (−), the EL-4 cells were either treated with TPA (+) or mock-treated (−). Then, CAT activities and *gpt* activities (parentheses) were measured.

The specific activity of the DNA was 3000 c.p.m./f mole in all probes. The unlabeled competitor DNAs were prepared similarly.

*Methylation Interference Analysis*

Methylation interference analysis was carried out as described previously (15). The probe DNAs were exactly the same as those described above, except that the probe DNAs were labeled by T4 kinase at the 5'-terminus of the coding or non-coding strand.

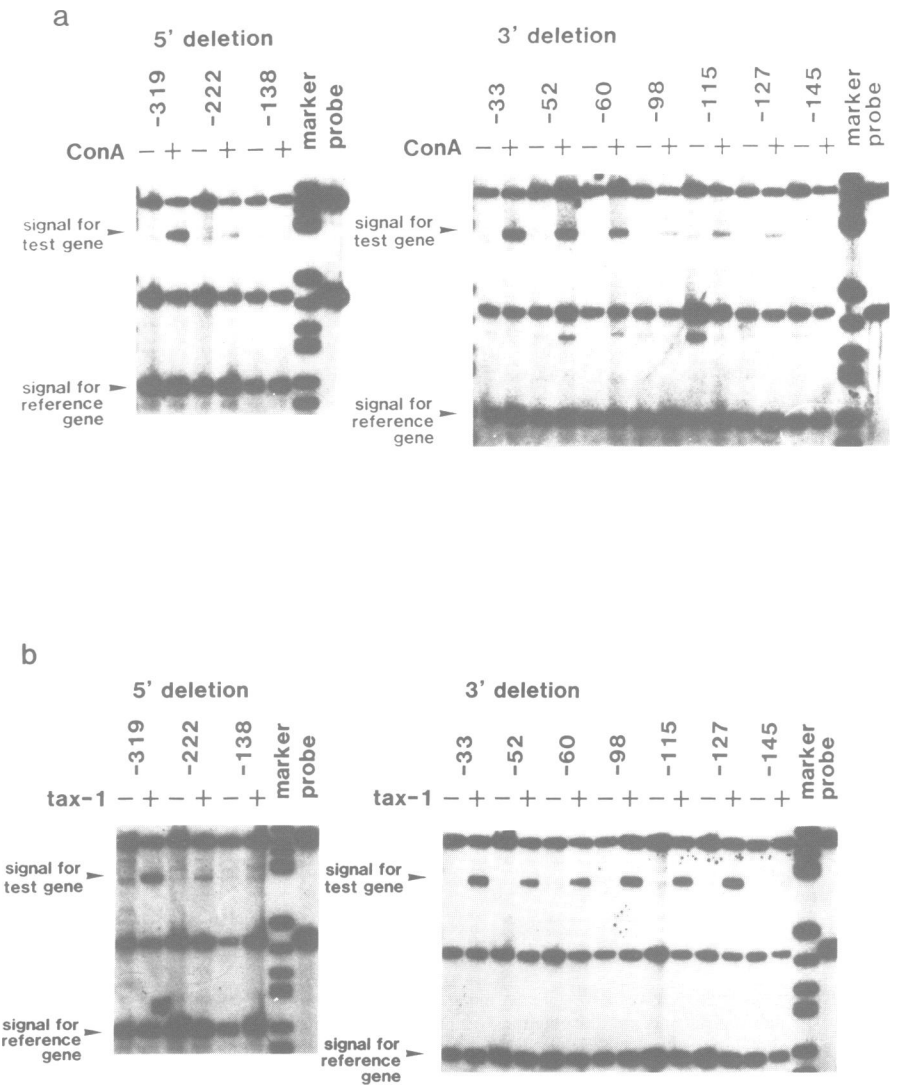


**Figure 2.** S1 mapping analysis of the various IL-2 deletion mutant gene transcripts in Jurkat cells. The genes were each cotransfected into Jurkat cells with a reference gene, pRSVtk. The cells were then treated with ConA (a). Alternatively, the vector DNAs were transfected with a *tax*-1 expression vector, pCDS (b) and the cells were not treated by mitogen. Assuming that the DNA transfection efficiency is 1% in Jurkat cells (5,6), the induced mRNA levels were 212 and 180 strands per cell in the mitogen-stimulated cells and the *tax*-1-expressing cells respectively in the case of pSI319cat. The transfection experiments were repeated at least three times and the results are highly reproducible.

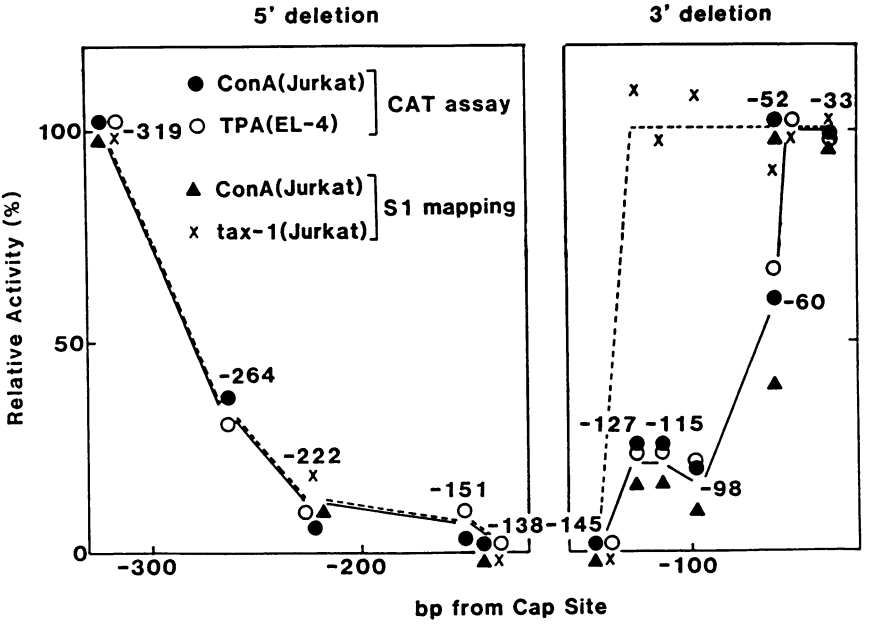


**Figure 3.** Summary of the expression of the IL-2 deletion mutant genes. The induced expression level of pSI319cat was taken as 100%.

## RESULTS

*Functional DNA sequences of the IL-2 promoter region*

In a previous report, it was shown that the mitogen-responsive promoter sequences reside within the 5'-flanking region of the human IL-2 gene (7, 8). More recently, similar results were reported for the mouse IL-2 gene (10). In order to dissect further the functional DNA sequence elements within the IL-2 promoter region extending from the CAP site to −319 (7), we constructed a series of 5'- or 3'-deletion mutants. These deletion mutants were fused to the human IFN-β promoter and bacterial chloramphenicol acetyltransferase (CAT) structural gene as the indicator gene (depicted in Fig. 1).

As shown in Fig. 1, the 5'-deletion mutant pSI319cat was highly inducible both in ConA-stimulated Jurkat and TPA-stimulated EL-4 cells, but as the deletion was extended to −222 (pSI222cat) the level of CAT expression was dramatically reduced. When the deletion was extended to −138, CAT activity was virtually undetectable. These results are in good agreement with previous reports (7, 8). Analysis of the 3'-deletion mutants revealed that the 3'-deletions which extended to −98 resulted in dramatic losses of inducible activity. Furthermore, inducibility was completely abrogated when the deletion was extended to −145. Essentially, the same DNA sequences seem to be required in both Jurkat and EL-4 cells (see also Fig. 3).

We next analyzed the mRNA transcripts by S1 mapping. As shown in Figure 2a, induction-specific RNA transcripts were generated from the CAP site of the IFN-β gene, as judged by the size of the protected probe DNA. As expected, the levels of such transcripts

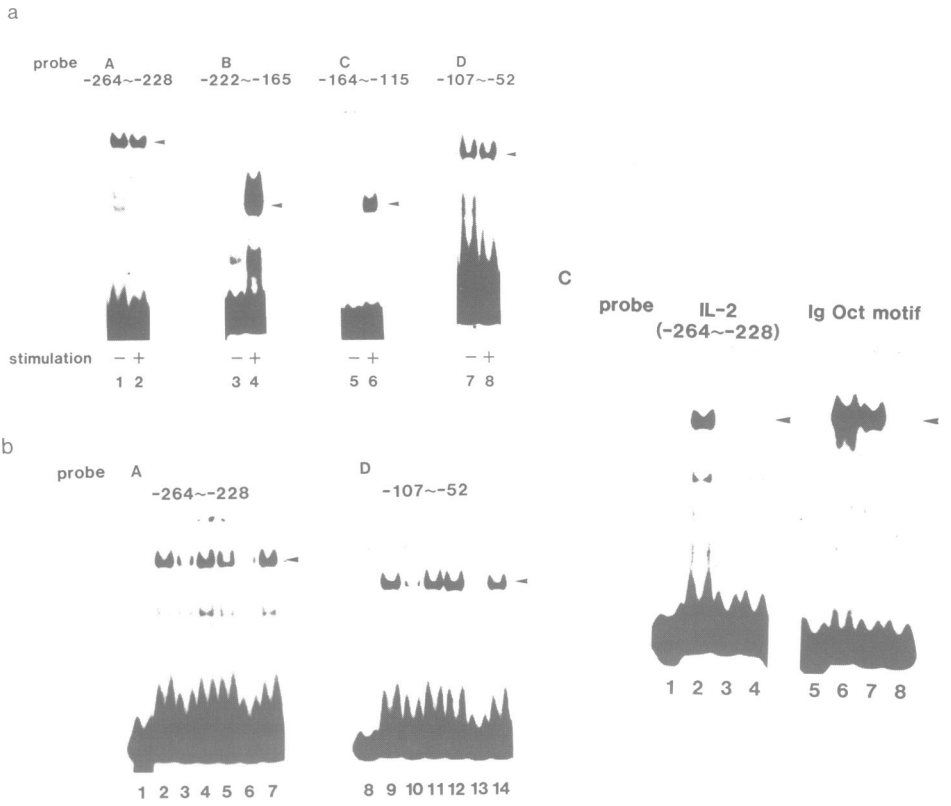


**Figure 4.** The binding of nuclear factors to the regulatory sequence elements of the IL-2 genes. (a) Gel-retardation assays for the detection of a factor that binds to IL-2 sequences. Nuclear extracts (10 $\mu$g) from unstimulated or mitogen-stimulated Jurkat cells (1 $\mu$g/ml PHA, 50 ng/ml TPA for 3 hr) were incubated with IL-2 DNA probe. Lane 1, 3, 5 and 7, extract from uninduced cells (−); lanes 2, 4, 6 and 8, extract from induced cells (+). (b) Gel-retardation assays using the IL-2 probe which encompasses from −228 to −264 (lanes 1−7) or from −52 to −107 (lanes 8−14). Nuclear extracts from unstimulated Jurkat cells were prepared and subjected to gel-retardation assay. Lanes 1 and 8: probe DNA; lanes 2 and 9: no competitor; lanes 3 and 10: 400 fold molar excess of unlabeled IL-2 DNA spanning from −228 to −264; lanes 4 and 11: 400 fold molar excess of unlabeled DNA spanning from −165 to −222; lanes 5 and 12: 400 fold molar excess of unlabeled DNA spanning from −115 to −164; lanes 6 and 13: 400 fold molar excess of unlabeled DNA spanning from −52 to −107; lanes 7 and 14: 400 fold molar excess of unlabeled DNA spanning from −257 to −301. (c) Gel-retardation assays using the IL-2 probe which encompasses from −228 to −264 (lanes 1−4) or the Ig Oct motif probe (lanes 5−8). Nuclear extracts from unstimulated Jurkat cells were prepared and subjected to gel-retardation assay. Lanes 1 and 5: probe DNA; lanes 2 and 6: no competitor; lanes 3 and 7: 400 fold molar excess of unlabeled IL-2 DNA spanning from −228 to −264; lanes 4 and 8: 400 fold molar excess of unlabeled Ig Oct motif DNA.

correlated with the respective CAT activities described above. These results suggest that the 5′-flanking sequence of the IL-2 gene consists of several functional regions extending from −52 to −98, −127 to −151, and −222 to −319, for the induced transcription of the gene in both Jurkat and EL-4 cells (Fig. 3).

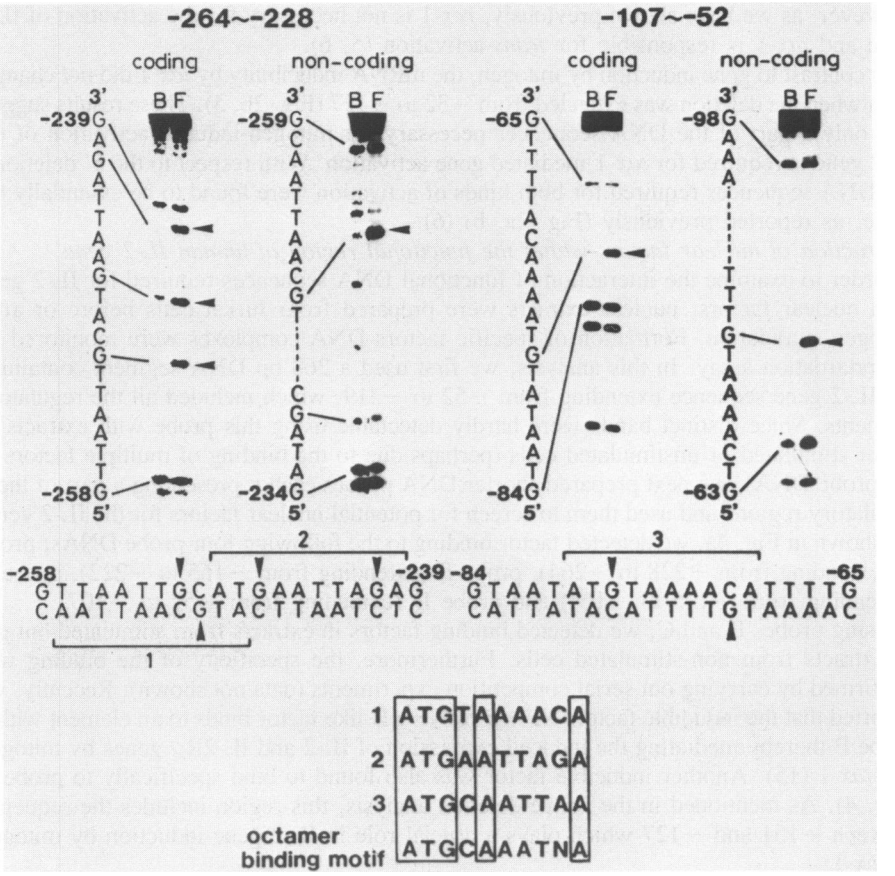


**Figure 5.** Methylation interference analysis for the factor contact regions of two IL-2 DNA elements. The left panel shows the analysis of the coding and non-coding strand of the IL-2 DNA probe spanning from −228 to −264, and the right panel shows the analysis of the DNA probe spanning from −52 to −107. The positions of methylated guanine residues that interfere with the binding of this factor are marked.

*Delimitation of the DNA sequences required for the IL-2 gene expression by HTLV-1-encoded tax-1*

Previous reports have demonstrated that the HTLV-1-encoded *tax*-1 transcriptionally activates the human IL-2 and IL-2Rα genes in Jurkat cells in the absence mitogenic stimulation (5, 6, 11). It has been shown that the DNA sequences in the 5′-flanking region of the IL-2 gene required for *tax*-1-mediated activation were indistinguishable from those required for mitogen-induced activation of the gene (6).

In order to study whether or not the same DNA sequences are required for the 3′ sequences in *trans*-activation by *tax*-1, the deletion mutants described above were each co-transfected with a *tax*-1 expression plasmid, pCDS (15, 21), into Jurkat cells. In addition to *tax*-1, the pCDS contains an open reading frame for viral regulatory protein *rex*-1.

However, as we have shown previously, *rex*-1 is not necessary for the activation of IL-2 gene and *tax*-1 is responsible for *trans*-activation (5, 6).

In contrast to gene induction by mitogen, the mRNA inducibility by *tax*-1 did not change, even when the deletion was extended from −32 to −127 (Fig. 2b, 3). These results suggest that only a part of the DNA sequences necessary for mitogen-induced activation of the IL-2 gene is required for *tax*-1-mediated gene activation. With respect to the 5′ deletions, the DNA sequences required for both kinds of activation were found to be essentially the same, as reported previously (Fig. 2a, b) (6).

*Interaction of nuclear factors within the functional region of human IL-2 gene*

In order to examine the interaction of functional DNA sequences required for IL-2 gene with nuclear factors, nuclear extracts were prepared from Jurkat cells before or after mitogen-stimulation. Formation of specific factors-DNA complexes were monitored by gel-retardation assay. In this analysis, we first used a 268 bp DNA segment containing the IL-2 gene sequence extending from −52 to −319, which included all the regulatory elements. Since distinct bands were hardly detectable using this probe with extracts of either stimulated or unstimulated cells (perhaps due to the binding of multiple factors to the probe DNA), we next prepared shorter DNA probes each representing a part of these regulatory regions and used them to screen for potential nuclear factors for the IL-2 gene. As shown in Fig. 4a, we detected factor binding to the following four probe DNAs; probe A (extending from −228 to −264), probe B (extending from −165 to −222), probe C (extending from −115 to −164) and probe D (extending from −52 to −107).

Using probes B and C, we detected binding factors in extracts from stimulated but not in extracts from non-stimulated cells. Furthermore, the specificity of the binding was confirmed by carrying out serial competition experiments (data not shown). Recently, we reported that the inducible factor, NF-$\varkappa$B or NF-$\varkappa$B-like factor binds to an element within probe B thereby mediating the induced expression of IL-2 and IL-2R$\alpha$ genes by mitogen and *tax*-1 (15). Another inducible factor was also found to bind specifically to probe C (Fig. 4). As mentioned in the above deletion analysis, this region includes the sequence between −151 and −127 which plays a crucial role in IL-2 gene induction by mitogen or *tax*-1.

Using probes A and D, we detected a shifted band in extracts of both stimulated and non-stimulated cells. Since the migration of these shifted bands were similar with either probe A or D, competition studies were carried out using unlabeled probe DNAs. In fact, binding of each factor was inhibited with unlabeled probe A or D, but not with other unlabeled probe DNAs (Fig. 4b). These observations suggest that the same factor(s) is binding to probe A and D. Similar observations have been reported by Durand et al. (12), however, the nature of this factor binding remained unclear. To identify the contact sites of this factor(s), methylation interference analysis was performed using these probe DNAs. As shown in Figure 5, the contact regions for the factor appear to be around −251 to −240 and −77 to −71 of the IL-2 gene. Interestingly, the contact region shows remarkable sequence similarity to the binding sites of octamer binding factors, such as Oct-1 or Oct-2 that binds to Ig gene promoter/enhancer elements (22). In this context, probe A may contain two such binding sites which overlap with each other (Fig. 5). In fact, the binding of this constitutive factor(s) was found to be strongly inhibited by a synthesized Ig gene segment containing the octamer motif (Fig. 4c). These results suggest that either Oct or Oct-like factor binds to the two IL-2 gene elements.

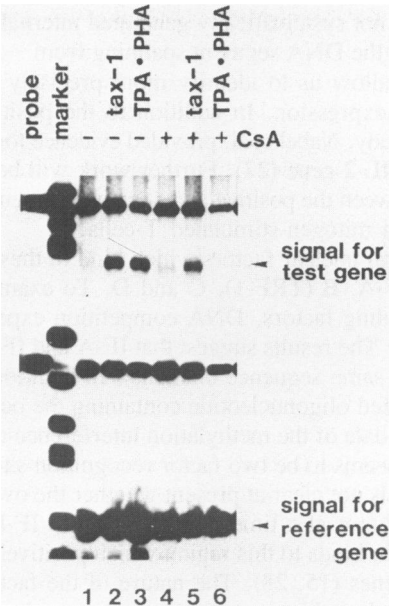


**Figure 6.** Cyclosporin-A inhibits mitogen-induced activation of the IL-2 gene, but not *tax*-1-mediated activation. The gene expression level was monitored by S1 analysis of the induced mRNA. Jurkat cells ($1.2\times10^7$ cells/sample) were transfected with the following plasmids: lanes 1, 3, 4 and 6, pSI319cat (20 μg), pBR322 (5 μg) and pRSVtk (5 μg); lanes 2 and 5, pSI319cat (20 μg), pCDS (5 μg) and pRSVtk (5 μg) in the presence (lanes 1–3) or absence (lanes 4–6) of CsA (1 μg/ml). After 24 hr, the cells were further treated with TPA (50 ng/ml) and PHA (1 μg/ml) (lanes 3 and 6) or mock-treated (lanes 1, 2, 4 and 5) for 4 hr.

*Cyclosporin A does not inhibit the tax-1-mediated activation of the IL-2 gene*

Cyclosporin A is a potent immunosuppressive agent that inhibits mitogen-activated transcription of the IL-2 gene (23–26). The above observations showing that distinct DNA segments are required in mitogen-induced and *tax*-1-induced IL-2 gene activation prompted us to examine whether CsA has differential effect in these two induction pathways. As shown in Fig. 6, CsA could inhibit PHA and TPA activation of the IL-2 gene expression in a transient-expression system. In contrast, CsA did not inhibit *tax*-1 mediated activation. These findings indicate that CsA selectively inhibits the IL-2 gene induction pathway(s) in mitogen-stimulated cells.

## DISCUSSION

We have previously shown that the 5′ boundary of the regulatory sequence for the induced human IL-2 gene expression is located between −319 and −264 from the cap site (7), and Durand et al. have also demonstrated that a 274-bp fragment extending from −52 to −326 of the IL-2 gene functions as an inducible enhancer (8). In the present study, the 5′-flanking region was dissected further by generating deletions extending from −33 to the upstream region. The results with both 5′ and 3′ deletion mutants (Fig. 1), together with our previous report (15), indicate the presence of at least four functional DNA elements within the 5′-flanking region, which play a positive role in mitogen-stimulated IL-2 gene

expression. Since we have not systematically generated internal mutations (such as linker scanning mutations) within the DNA segment spanning from −33 to −319, we think that a detailed dissection may allow us to identify more precisely the multiple *cis*-elements controlling the IL-2 gene expression. In addition to the positive regulatory regions as revealed from the present study, Nabel et al. provided evidence for the presence of a negative element within the human IL-2 gene (27). Further work will be required to elucidate the mechanism of interplay between the positive and negative elements in the overall regulation of IL-2 gene expression in mitogen-stimulated T-cells.

We have identified several nuclear factors which bind to these elements. These factors were termed tentatively IF-A, B (TRF-1), C and D. To examine the relative sequence specificities with these binding factors, DNA competition experiments were carried out using these DNA elements. The results suggest that IF-A and IF-D are probably identical, as they appear to bind the same sequence elements. In addition, these two factors were found to bind to a synthesized oligonucleotide containing the octamer motif of the human Ig enhancer (Fig. 4c). The data of the methylation interference analysis with IF-A is quite interesting, because there seems to be two factor recognition sites which partially overlap with each other (Fig. 5). It is not clear at present whether the overlapping region basically limits the binding of one factor at a time. With respect to IF-B, it has been shown that NF-$\varkappa$B or NF-$\varkappa$B-like factor binds to this region which positively regulates the expression of the IL-2 and IL-2R$\alpha$ genes (15, 28). The nature of the factor binding to IF-C is yet the subject of more detailed studies.

While this work was in progress, Durand et al. reported the presence of five nuclear factors which interact with the regulatory elements of the IL-2 gene (10). Four of these factors might be identical to the factors presented in this study. Moreover, on the basis of DNase I foot printing analysis, Serfling et al. reported that many more factors including AP-1 and AP-3 bind to the 5′ regulatory region of the mouse IL-2 gene (10). It is important to examine as to what extent those factor bindings are functionally important for IL-2 gene expression.

Our findings demonstrate that the maximum activation of the promoter by *tax*-1 does not require the DNA sequence extending from −52 to −127, which is essential for the activation of the gene by mitogens. The corresponding region contains a binding site for an Oct-like constitutive factor(s), identical or similar to another constitutive factor which binds to a further upstream region (Fig. 5). In addition, similar requirements for mitogen-stimulated activation of the gene were observed in the mouse T-cell line, EL-4, but *tax*-1-mediated activation was absent (6, data not shown). It has been shown that *tax*-1 per se does not manifest specific DNA binding properties (6, 29, 30). Thus, these data imply that *tax*-1 might interact with pre-existing specific DNA binding proteins and/or it induces a gene product(s) which is able to interact with the regulatory region either singly or in association with *tax*-1 in the human T-cell line. In such events, the transcriptional activation of the IL-2 gene would occur without the transcription factor associated to the DNA sequence extending from −52 to −127.

The effect of CsA, a potent immunosuppressive compound, was studied in order to distinguish activation of IL-2 gene by mitogens or by *tax*-1. It is known that CsA inhibits activation of IL-2 gene by mitogens (23−26) and by calcium ionophore, A23187 (31). In contrast, CsA had no effect on *tax*-1-mediated activation of the IL-2 gene. It is interesting to note that the above factor binding profiles to the IL-2 regulatory regions using extracts from stimulated or unstimulated cells were not affected dramatically in the presence of

CsA (data not shown), suggesting that CsA has little or no effects on factor binding to the DNA sequences. At present, we cannot rule out the possibility as to whether the binding of other factors not detected in our study is affected by CsA. Whatever the mechanisms, the observations suggest the existence of a unique pathway of gene activation by *tax*-1 that may be different from the pathway triggered by other extracellular activation signals. Since *tax*-1 is localized in the nucleus (29), it is also likely that the protein bypasses the extracellular signal pathways. The observations made in this study with *tax*-1 hint to the presence of multiple pathways for IL-2 gene expression.

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## REFERENCES

1. Smith, K. A. (1988) Science **240,** 1169–1176.
2. Greene, W. C. and Leonard, W. J. (1986) Ann. Rev. Immunol. **4,** 69–96.
3. Taniguchi, T. (1988) Ann. Rev. Immunol. **6,** 439–464.
4. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. and Taniguchi, T. (1989) Science **244,** 551–556.
5. Inoue, J., Seiki, M., Taniguchi, T., Tsuru, S. and Yoshida, M. (1986) EMBO J. **5,** 2883–2888.
6. Maruyama, M., Shibuya, H., Harada, H., Hatakeyama, M., Seiki, M., Fujita, T., Inoue, J., Yoshida, M. and Taniguchi, T. (1987) Cell **48,** 343–350.
7. Fujita, T., Shibuya, H., Ohashi, T., Yamanishi, K. and Taniguchi, T. (1986) Cell **46,** 401–407.
8. Durand, D. B., Bush, M. R., Morgan, J. G., Weiss, A. and Crabtree, G. R. (1987) J. Exp. Med. **165,** 395–407.
9. Cross, S. L., Feinberg, M. B., Wolf, J. B., Holbrook. N. J., Wong-Staal, F. and Leonard, W. J. (1987) Cell **49,** 47–56.
10. Serfling, E., Barthelmäs, R., Pfeuffer, I., Schenk, B., Zarius, S., Swoboda, R., Mercurio, F. and Karin, M. (1989) EMBO J. **8,** 465–473.
11. Shibuya, H., Harada, H., Maruyama, M., Fujita, T., Seiki, M., Inoue, J., Yoshida, M., Hatakeyama, M. and Taniguchi, T. (1987) in Molecular Basis of Lymphokine Action, eds. Webb, D. R., Pierce, C. W. and Cohen, S., p161–169, The Humana Press, New York.
12. Durand, D. B., Shaw, J. P., Bush, M. R., Replogle, R. E., Belagaje, R. and Crabtree, G. R. (1988) Mol. Cell. Biol. **8,** 1715–1724.
13. Böhnlein, E., Lowenthal, J. W., Siekevitz, M., Ballard, D. W., Franza, B. R. and Greene, W. C. (1988) Cell **53,** 827–836.
14. Leung, K. and Nabel, G. J. (1988) Nature **333,** 776–778.
15. Shibuya, H., Yoneyama, M. and Taniguchi, T. (1989) Int. Immunol. **1,** 43–49.
16. Fujita, T., Shibuya, H., Hotta, H., Yamanishi, K. and Taniguchi, T. (1987) Cell **49,** 357–367.
17. Gorman, C., Moffat, L. and Howard, B. (1982) Mol. Cell. Biol. **2,** 1044–1051.
18. Fujita, T., Miyamoto, M., Kimura, Y., Hammer, J and Taniguchi, T. (1989) Nucleic Acids Res. **17,** 3335–3346.
19. Fujita, T., Sakakibara, J., Sudo, Y., Miyamoto, M., Kimura, Y. and Taniguchi, T. (1988) EMBO J. **7,** 3397–3405.
20. Müllar, M. M., Ruppert, S., Schaffner, W. and Matthias, P. (1988) Nature **336,** 544–551.
21. Doi, T., Hatakeyama, M., Itoh, S. and Taniguchi, T. (1989) EMBO J. **8,** 1953–1958.
22. Robertson, M. (1988) Nature **336,** 522–524.
23. Krönke, M., Leonard, W. J., Depper, J. M., Arya, S. K., Wong-Staal, F., Gallo, R. C., Waldmann, T. A. and Greene, W. C. (1984) Proc. Natl. Acad. Sci. USA **81,** 5214–5218.

24. Elliott, J. F., Lin, Y., Mizel, S. B., Bleackley, R. C., Harnish, D. G. and Peatkau, V. (1984) Science **226,** 1439–1441.
25. Granelli-Piperno, A., Inaba, K. and Stainman, R. M. (1984) J. Exp. Med. **160,** 1792–1802.
26. Bickel, M., Tsuda, H., Amstad, P., Evequoz, V., Mergenhagen, S. E., Wahl, S. M. and Pluznik, D. H. (1987) Proc. Natl. Acad. Sci. USA **84,** 3274–3277.
27. Nabel, G. J., Gorka, C. and Baltimore, D. (1988) Proc. Natl. Acad. Sci. USA **85,** 2934–2938.
28. Hoyos, B., Ballard, D. W., Böhnlein, E., Siekevitz, M. and Greene, W. C. (1989) Science **244,** 457–460.
29. Yoshida, M. and Seiki, M. (1987) Ann. Rev. Immunol. **5,** 541–559.
30. Greene, W. C. Böhnlein E. and Ballard D. W. (1989) Immunol. Today **10,** 272–278.
31. Shevach, E. M. (1985) Ann. Rev. Immunol. **3,** 397–423.

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