A Regulatory Element in the Human Interleukin 2 Gene Promoter Is a Binding Site for the Zinc Finger Proteins Sp1 and EGR-1*

(Received for publication, April 27, 1995, and in revised form, July 17, 1995)

Christine Skerka, Eva L. Decker, and Peter F. Zipfel†
From the Department of Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Strasse 74, D-20359 Hamburg, Germany

Activation of the interleukin 2 (IL-2) gene after antigen recognition is a critical event for T cell proliferation and effector function. Prior studies have identified several transcription factors that contribute to the activity of the IL-2 promoter in stimulated T lymphocytes. Here we describe a novel regulatory element within the IL-2 promoter located immediately upstream of the nuclear factor of activated T cell (NFAT) domain. This region (termed the zinc finger protein binding region (ZIP)) serves as binding site for two differently regulated zinc finger proteins: the constitutively expressed transcription factor Sp1 and the inducible early growth response protein EGR-1. In unstimulated cells which do not secrete IL-2, only Sp1 binds to this region, while in stimulated IL-2 secreting cells the inducible EGR-1 protein recognizes this element. In Jurkat T cells, the ZIP site serves as an activator for IL-2 gene expression, and a combination of ZIP and NFAT binding sites is required for maximal IL-2 promoter activity. These results suggest a critical role of the ZIP site for IL-2 promoter activity.

The Journal of Biological Chemistry Vol. 270, No. 38, Issue of September 22, pp. 22500–22506, 1995

*This work was supported by Deutsche Forschungsgemeinschaft (DFG) Grant Zi 432/2-1. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed. Tel.: 0049-40-3-11-82-472; Fax: 0049-40-3-11-82-400.

The abbreviations used are: EGR, early growth response genes; NFAT, nuclear factor of activated T cells; ZIP, zinc finger protein binding region; AP-1, activator protein 1; IL-2, interleukin 2; bp, base pair(s); PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate.

T cell activation by antigenic peptides in combination with antigen presenting cells induces a cascade of metabolic events which result in the transcriptional activation of a large number of different genes (1, 2). Among the transcripts induced in the “immediate early phase,” i.e. about 15 min following the activating stimulus in the absence of denovo protein synthesis, are a large number of transcription factors. These factors are considered to regulate the induction of genes which are transcribed in a second phase, i.e. the “early phase.” A family of genes, termed “early growth response genes” (EGR), which encode DNA-binding proteins with almost identical zinc finger domains is induced directly in the immediate early phase. The four EGR genes, termed EGR-1 (3), EGR-2 (4), EGR-3 (5, 6), and EGR-4/p133 (5, 7) are coordinately regulated. No transcript is detected in quiescent, nonproliferating T lymphocytes, nor in resting, serum-deprived fibroblasts; however, all genes are transiently induced in a variety of cells upon mitogenic stimulation (8–13). Although the four proteins are closely related within their zinc finger domains, their flanking regions are much less conserved. As already expected from the high amino acid homology of their zinc finger domains, in vitro experiments confirmed binding of all four proteins to the same target sequence, i.e. GCG G/TGG GCG (4–6, 13–18). Despite binding to the identical sequence, the amino acid differences in the flanking regions suggest distinct biological functions of the four proteins.

As primary response factors, EGR-1 to EGR-4 are candidates for the regulation of distal gene expression. Based on their later time course of induction, cytokine genes are likely target genes for EGR regulation in T lymphocytes. In order to provide evidence for such a regulation, we searched for EGR binding motifs in the promoter region of human cytokine genes. This approach resulted in the identification of putative EGR binding motifs within the promoter of the human IL-2 and tumor necrosis factor α genes. The EGR motif in the tumor necrosis factor α promoter has recently been characterized as a binding site for the EGR-1 protein (19).

Transcriptional activation of the IL-2 gene and IL-2 secretion are essential steps for T cell proliferation, differentiation, and effector functions. IL-2 gene regulation is analyzed extensively, and several regulatory elements and their corresponding binding proteins have been identified in both the human and the mouse IL-2 promoter (1, 20–24). In particular, a critical role for IL-2 gene expression has been demonstrated for the nuclear factor of activated T cells (NFAT). This complex factor assembles only in the nucleus of activated T cells and is essential for transcriptional induction of the IL-2 gene (25–27). The NFAT binding region includes a purine-rich stretch and an AP-1 site. Binding of members of the fos and jun gene family to the AP-1 site has been demonstrated (28), and two factors (termed NFATc and NFATp) which bind to the purine-rich region directly upstream of the AP-1 site have been cloned recently (25, 29).

The putative EGR recognition element in the human IL-2 promoter is located directly upstream of the NFAT site. We asked whether this site serves as a binding site for nuclear proteins and whether it participates in transcriptional regulation of IL-2 gene expression. Here we demonstrate that this G-rich domain is a binding site for the two zinc finger proteins Sp1 and EGR-1. In stimulated Jurkat T cells, this EGR-1 binding element is a novel and important regulatory element for IL-2 gene activation.

EXPERIMENTAL PROCEDURES

Insect Cell Culture—Spodoptera frugiperda cells (Sf9) were grown at 27 °C in monolayer culture, in Grace’s medium (BioWhittaker, Inc.) supplemented with 10% fetal bovine serum, streptomycin (0.2 μg/ml), penicillin (0.2 units/ml), and fungizone (250 ng/ml) or in fetal bovine serum-free Express-medium (BioWhittaker).

Expression of Recombinant Proteins in the Baculovirus System—
Plate-purified EGR-1 or AT133/EGF-4 baculoviruses were prepared by standard methods (30) and were used to infect Sf9 insect cells, cultivated in Grace's medium. A multiplicity of infection of 5 was used. After 70 h of incubation, cells were lysed by three freeze-thaw cycles, and whole-cell extracts were prepared as described (31). The lysis buffer (10 mM sodium phosphate, pH 7.4, 0.5 mM dithiothreitol, 400 mM KCl, 10 μM ZnCl₂, 10% glycerol) contained proteinase inhibitors phenylmethylsulfonyl fluoride (1 mM), leupeptin (5 μg/ml), and antipain (5 μg/ml).

IL-2 Reporter Constructs—Specific primers were used for polymerase chain reaction amplification of the indicated regulatory elements using a 417-bp fragment of the human IL-2 promoter region. Amplified fragments were ligated into the Kpn I and Sac I restriction sites of the pGL2-Basic vector to yield plasmid pCILuc1. Plasmid pCILuc2 was constructed in the same way using primers eu9 and eu6 (tttggatcc AGGAATTC AGAAGTTTAC, position 260 to 63 of the human IL-2 promoter, the ZIP site). The resulting fragments were excised by Kpn I and Sac I, respectively, and ligated with pPacSp1 covering the 696 C-terminal amino acids of Sp1 (kindly provided by J. Benezra, The Rockefeller University, New York, NY). Plasmids pCILuc3 and pCILuc4 were constructed in the same way using primers eu9 and eu6 (tttggatcc AGGAATTC AGAAGTTTAC, position 260 to 63 of the human IL-2 promoter, the ZIP site). The resulting fragments were excised by Kpn I and Sac I, respectively, and ligated with pPacSp1 covering the 696 C-terminal amino acids of Sp1 (kindly provided by J. Benezra, The Rockefeller University, New York, NY).

In order to prove whether the G-rich element in the human IL-2 gene promoter (Fig. 1A) serves as binding site for nuclear proteins, a corresponding oligonucleotide was used for

**RESULTS**

Binding of Nuclear Proteins to the ZIP Element of the Human IL-2 Gene—In order to prove whether the G-rich element in the human IL-2 gene promoter (Fig. 1A) serves as binding site for nuclear proteins, a corresponding oligonucleotide was used for
NFATm site (termed GC7) blocked formation of the lower complex (Fig. 2). Specific antiserum identifies Sp1 in the upper complex, present in unstimulated and in stimulated J urkat cells, and EGR-1 in the lower complex of extract prepared from stimulated J urkat cells (lanes 3 and 4). Antibody binding to the protein complexes results in a “supershift” and in a reduction in mobility. Competition experiments confirm different binding specificities of the two complexes. Competition with unrelated oligonucleotides (NFAT and NFATm) did not affect binding, while competition with an oligonucleotide displaying an EGR consensus site (GC7) specifically interfered with formation of the lower EGR-1-containing complex.

As de novo protein biosynthesis is critical for IL-2 gene induction, we investigated whether both protein complexes were pre-existing in unstimulated cells. In nuclear extracts prepared from unstimulated J urkat T cells, only the upper complex was observed (Fig. 3, lane 1), while both complexes were detected in extracts prepared from stimulated cells (lane 2). Specific antiserum was used for further differentiation and identification of the two protein complexes. Sp1 and EGR-1 antiserum specifically interfered with formation of the two complexes. Antiserum directed against EGR-1 specifically altered migration of the lower complex (Fig. 3, lane 3), indicating that EGR-1 gives rise to the inducible protein complex. These results indicated that Sp1 binds to the ZIP site of the human IL-2 promoter. Previously, specific and exclusive binding to G-rich consensus sequences of EGR-1 (GGG GGG GGG) and Sp1 (GGG GGG GGG) has been demonstrated for EGR-1 and the mouse homolog Zif 268, for Krox 20, the mouse homolog of EGR-2 as well as for Sp1 (14, 15, 18). Taken together the results suggest that the G-rich region of the human IL-2 promoter serves as a binding site for the zinc finger proteins Sp1 and EGR-1. Therefore, this region was termed the zinc finger protein (ZIP) binding site.

Regulation and Binding of the EGR-1 and Sp1 Proteins—To examine the expression of the ZIP binding factors in more detail, a time course of induction was determined on the mRNA and on the protein level. Sp1 mRNA was found synthesized in unstimulated (Fig. 4A, upper panel, lane 1) as well as in stimulated (lanes 2–6) J urkat T cells, without significant modulation in expression levels. In contrast, EGR-1 messenger RNA was not expressed in unstimulated cells, but was induced 30 min after stimulation (Fig. 4A, lower panel). mRNA transcription was independent of protein biosynthesis (cycloheximide (Chx), lanes 7–9) and confirmed EGR-1 as an immediate early gene in T cell activation (2, 33).

Similarly, binding assays of the two proteins paralleled the results obtained by Northern blotting. Sp1 protein binding to the ZIP site was constitutive, equal levels were detected in unstimulated cells and during the course of stimulation. Again the product of the immediate early response gene EGR-1 was

3 C. Skerka and P. F. Zipfel, unpublished data.
transiently expressed, and binding was detected 1 and 2 h after stimulation (Fig. 4B). Later time points did not show any EGR-1 protein complex formation (lanes 5 and 6). Thus, constitutive expression of Sp1 and transient synthesis of EGR-1 following cell stimulation was detected on the RNA level and in the protein binding assays. The fact that no additional band was detected when both proteins Sp1 and EGR-1 were present in the same nuclear extract suggests that EGR-1 competes with Sp1 for binding to the ZIP site and replaces Sp1.

Binding Specificities of EGR-1 and pAT133/EGR-4—We asked whether the lower complex was represented by a single EGR-1 protein or by a complex of proteins including EGR-1. Determining the binding of recombinant, baculovirus-expressed EGR-1 protein to the ZIP oligonucleotide resulted in one specific shifted band (Fig. 5, lane 1). This complex showed the same binding specificities as the induced complex detected in Jurkat cells, as it was competed with unlabeled ZIP oligonucleotides (compare Fig. 5, lane 3, with Fig. 2, lane 3), but not with an unrelated NFAT oligonucleotide (compare Fig. 5, lane 2, with Fig. 3, lane 5). In addition, antibodies raised against EGR-1, but not EGR-3 antisera, abolished binding of the recombinant protein (Fig. 5, lanes 4 and 5). Thus, binding of recombinant EGR-1 seems to be independent of additional T cell-specific transcription factors. Upon activation of peripheral blood T lymphocytes and Jurkat T cells, transcription of the four known EGR genes is simultaneously and coordinately induced (7), and binding to the consensus site (GC7) has been demonstrated in vitro for the corresponding proteins (4–6, 13–18). As the ZIP sequence differs from the known EGR consensus site, we addressed the question of whether other EGR proteins could bind to this promoter element. No binding of baculovirus-expressed AT133/EGR-4 protein was detected (Fig. 5, lane 6), although the recombinant AT133/EGR-4 protein recognized the ATG oligonucleotide, which includes a consensus site for the EGR proteins (lane 7).

Regulation of Gene Expression—The in vivo significance of...
the ZIP site on IL-2 gene expression was analyzed by transfection experiments. Various hybrid plasmid constructs containing the ZIP and NFAT sites with a regular or minimal IL-2 promoter were linked to the luciferase (Luc) gene (Fig. 6A). Upon transient transfection into Jurkat T cells, the individual constructs responded differently to T lymphocyte activation signals (Fig. 6B). The complete IL-2 reporter gene construct pCILuc1 (−366 to +51) (Fig. 6A) displayed regulatory activities characteristic for the endogenous IL-2 gene: it was inactive in unstimulated cells, but luciferase activity was induced 96-fold by stimulation with PHA and PMA. This induction was abrogated in the presence of cyclosporin A. The minimal activity obtained with the previously identified binding sites for Oct-1, NF-kB, AP-1, CD28RE, NRE, and Ets (for a review, see Ref. 24), shows the importance of a combination of the ZIP and the NFAT sites for IL-2 gene induction (Fig. 6B). The high activity obtained with the NFAT-containing construct (pCILuc2) is in agreement with previous results (34) and underlines the critical role of NFAT for IL-2 gene induction.

A single ZIP site (pCILuc3) increased promoter activity 3-fold and enhanced promoter activity in combination with the NFAT site (pCILuc1). To rule out regulatory effects by transcription factors binding between the NFAT site and position −63, the transcriptional role of the NFAT or ZIP site was also tested in front of a minimal IL-2 promoter (pMILuc4; −63 to +51). A single NFAT or ZIP element (pMILuc2 and pMILuc3) increased activity of the minimal promoter pMILuc4, 2.2- and 4.2-fold, respectively (Fig. 6B and Table I). The 20-fold increase in activity obtained by a combination of one ZIP with a single NFAT site highlights the role of the ZIP and NFAT binding factors in IL-2 gene expression and suggests synergism of action.

**DISCUSSION**

The current study identified a novel protein binding region in the human IL-2 gene promoter, which has a regulatory function in the early phase of T lymphocyte activation. Two zinc finger proteins Sp1 and EGR-1 were shown to bind to this element. In unstimulated and in stimulated Jurkat T cells, the ubiquitous transcription factor Sp1 binds to this region, while in activated cells the transiently induced EGR-1 protein binds this element. Due to binding of two differently regulated zinc finger proteins, this binding site was termed the zinc finger protein binding region (ZIP). By transfection experiments, a regulatory function of the ZIP site for IL-2 gene induction was demonstrated in Jurkat T cells. In PHA/PMA-stimulated cells, the ZIP site activated gene expression, particularly in combination with the NFAT region.

The ZIP site seems conserved in evolution as a highly related element is located in the same orientation in the mouse IL-2 gene promoter (position −298 to −290) immediately upstream of the NFAT binding site (Fig. 7). The conservation of the ZIP and NFAT domains in sequence and in location in both the human and the mouse IL-2 gene promoter is in agreement with the proposed role of this element in IL-2 gene regulation.

Although the ZIP site of the human IL-2 promoter does not display a typical binding site for Sp1 or EGR-1 (Fig. 1), both zinc finger proteins bind to this site. However, it has been demonstrated that Sp1 does not bind to the EGR consensus sequence (35—38), and, similarly, no binding of EGR-1 to the Sp1 consensus sequence was observed (14, 15). Despite this specificity of binding to the corresponding consensus sequences, binding to a range of different sites has been described for Sp1 as well as for EGR-1 (14, 39, 40). We conclude that there is exclusive binding of either Sp1 or EGR-1 to the ZIP site, as the mobilities of the factors remained unchanged, when both proteins were present in the nuclear extract at the same time (Fig. 3). The unchanged mobility of both complexes are also in agreement with this explanation. Overlapping EGR-1 and Sp1 binding sites as described here for the IL-2 gene promoter seem to be of general physiological relevance, as similar regions were identified recently in the promoters of the EGR-1 (15), the tumor necrosis factor α (19), the mouse adenosine deaminase (39), and the mouse thrombospondin 1 gene (40). Thus, competitive binding of these two distinct zinc finger proteins seems to play a critical role in gene regulation. Further experiments will clarify whether other Sp1 elements, found in a variety of gene promoters, can serve as binding sites and regulatory domains for inducible EGR proteins.

For the ZIP element of the IL-2 promoter, we could demonstrate preferential binding of EGR-1 but not of AT133/EGR-4 (Fig. 3). This is the first time that different binding specificities are reported for two members of this protein family. Although all four members of the EGR zinc finger gene family are coordinately induced upon mitogenic stimulation of T cells, binding of the individual proteins to their target sequences seems tightly regulated. Specificity of binding may be regulated by binding to slightly modified target sequences or by interactions with factors binding in close proximity of the identified binding region.

The significance of the ZIP site on IL-2 gene expression was demonstrated in vivo by transfection experiments using plasmid constructs containing the ZIP and NFAT site with a regular or minimal IL-2 promoter (Fig. 6A). The complete IL-2 reporter gene construct pCILuc1 displayed regulatory activities characteristic of the endogenous IL-2 gene, and induction was completely abrogated in the presence of cyclosporin A. The previously identified binding sites for Oct-1, NF-kB, AP-1, CD28RE, NRE, and Ets are all included in construct pCILuc4. However, the low activity obtained with this construct demonstrates that these sites alone have a minimal effect on transcription. The high activity obtained with the NFAT-containing construct (pCILuc2) demonstrates the important role of this regulatory element for IL-2 gene induction in T cells. A single ZIP element (pCILuc3) increased promoter activity; however, in contrast with the regular IL-2 promoter, the activating effect of a single NFAT region was more pronounced than that of a single ZIP site. The increase in activity observed with a combination of the two sites (pCILuc1) suggests an additive effect of the binding proteins.

A regulatory activity of the ZIP and NFAT elements and a possible interaction of the binding factors are confirmed from the minimal IL-2 promoter constructs. Regulatory effects by transcription factors binding to the identified sites located between the NFAT site and position −63 were ruled out by analyzing the NFAT or ZIP sites in front of a minimal IL-2 promoter (pMILuc4; −63 to +51). A single NFAT or ZIP element (pMILuc2 and pMILuc3) increased activity of the minimal promoter pMILuc4, 2.2- and 4.0-fold, respectively (Fig. 6B). The 20-fold increase in activity obtained by an IL-2 promoter element including the NFAT and the ZIP site, highlights the role of the ZIP and NFAT binding factors in IL-2 gene expression and suggests synergism of action.

**Binding of the zinc finger protein EGR-1 to the ZIP site was demonstrated in stimulated Jurkat cells, suggesting that EGR-1 protein functions as a positive regulator of IL-2 gene transcription. Previous studies have shown that EGR-1 can function equally well as an activator and repressor of gene expression (5, 14, 15, 19, 39, 40), and different functional domains have been localized within the protein (41). The close proximity of the ZIP and NFAT sites in the IL-2 promoter in**
combination with the experimental results suggests an interaction between their binding factors. Several proteins which are regulated differently have been shown to bind to the NFAT element (42, 43). NFAT is composed of a pre-existing cytoplasmic component (NFATc/p) that translocates into the nucleus upon T cell activation (25, 29, 44). There it interacts with the ubiquitous, newly synthesized AP-1 component to form a protein complex. Neither the two recently cloned NFAT proteins (NFATp and NFATc) nor EGR-1 display a common motif indicative for this kind of interaction. Additional experiments aimed at describing the interaction of these transcription factors will contribute to an understanding of their synergism of action.

Fig. 6. The ZIP and NFAT sites regulate gene expression. A, schematic representation of the IL-2/luciferase gene hybrids used for transient transfections. A 366-bp fragment of the IL-2 gene promoter and deletions thereof were fused to the firefly luciferase gene (Luc). Constructs pCILuc1-4 include a regular IL-2 promoter (position -253 to +51), while constructs pMILuc1-4 have the tested regulatory elements linked to a minimal IL-2 promoter (-63 to +51). The ZIP and NFAT binding regions (compare Fig. 1A) are indicated. For construction details, see "Experimental Procedures." B, Jurkat T cells were transfected with the indicated plasmids by electroporation. After 24 h, the transfected cells were stimulated with PHA (1 μg/ml) and PMA (25 ng/ml), and, 24 h later, cells were lysed and luciferase activity of the cell lysate was determined. Luciferase activity obtained with constructs (pCILuc1 and pMILuc1), which include a single ZIP and a single NFAT site, was set at 100%. Luciferase activity of each set of constructs is shown relative to the activity of construct pCILuc1 or pMILuc1. Each column represents the mean of five independent experiments, and standard deviations are indicated by bars.
Our data suggest that the zinc finger proteins Sp1 and EGR-1 are part of this transcriptional machinery which regulate the induction of the T cell growth factor IL-2.

Acknowledgments—We thank R. Tjian for providing plasmid pPaCSp1, Uli Siebenlist for the human IL-2 promoter region, and J.achim Clos for critical reading of the manuscript.

REFERENCES

1. Crabtree, G. R. (1989) Science 243, 355–361
2. Zipfel, P. F., Irving, S. G., Kelly, K., and Siebenlist, U. (1989) Mol. Cell. Biol. 9, 1041–1045
3. Suggs, S. V., Katowitz, J. L., Tsai-Morris, C., and Sukhatme, V. P. (1990) Nucleic Acids Res. 18, 4283–4288
4. Joseph, L. J., Le Beau, M. M., and Sukhatme, V. P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7164–7168
5. Patwardhan, S., Gashler, A., Siegel, M. G., Chang, L. C., Joseph, L. J., Shows, T. B., Le Beau, M. M., and Sukhatme, V. P. (1991) Oncogene 6, 917–928
6. Mages, H. W., Stammlinger, T., Rilke, O., Bravo, R., and Kroczek, R. A. (1993) Intern. Immunol. 5, 63–70
7. Müller, H.-J., Skerka, C., Bialonski, A., and Zipfel, P. F. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10079–10083
8. Sukhatme, V. P., Kartha, S., Toback, F. G., Taub, R., Hoover, R. G., and Tsai-Morris, C.-H. (1987) Oncogene Res. 1, 343–355
9. Milbrandt, J. (1987) Science 238, 797–799
10. Christy, B. A., Lau, L. F., and Nathans, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7857–7861
11. Lemaire, P., Revellant, O., Bravo, R., and Charnay, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4691–4695
12. Chavrier, P., Zerial, M., Lemaire, P., Almandral, J., Bravo, R., and Charnay, P. (1990) EMBO J. 7, 29–35
13. Crosby, S. D., Puch, J. J., Sirmburger, K. S., Fahmer, T. J., and Milbrandt, J. (1991) Mol. Cell. Biol. 11, 3835–3841
14. Christy, B., and Nathans, D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8737–8741
15. Cao, X., Mahendran, R., Guy, G. R., and Tan, Y. H. (1993) J. Biol. Chem. 268, 16949–16957
16. Pavletich, N. P., and Pabo, C. O. (1991) Science 250, 809–817
17. Lemaire, P., Vesque, C., Schmitt, J., Stunnenberg, H., Frank, R., and Carnay, P. (1990) Mol. Biol. 10, 3456–3467
18. Chavrier, P., Vesque, C., Galliot, B., Vigneron, M., Dolié, P., Dubole, D., and Charnay, P. (1990) EMBO J. 9, 1209–1218
19. Krämer, B., Meichle, A., Hensel, G., Charnay, P., and Krönke, M. (1994) Biochem. Biophys. Acta 1219, 413–421
20. 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
21. Kamps, M. P., Corcoran, L., Lebowitz, J. H., and Baltimore, D. (1990) Mol. Cell. Biol. 10, 5464–5472
22. Graneli-Piperno, A., and Nolan, P. (1991) J. Immunol. 147, 2734–2739
23. Garray, P. A., Chen, D., von Rothenburg, E., and Wold, B. J. (1994) Mol. Cell. Biol. 14, 2159–2169
24. Serriono, E., Avdal, A., and Neumann, M. (1995) Biochim. Biophys. Acta, in press
25. Northrop, J. P., Ho, S. N., Chen, L., Thomas, D. J., Timmerman, L. A., Nolan, G. P., Admon, A., and Crabbe, G. R. (1994) Nature 369, 497–502
26. Rao, A. (1994) Immunity. 15, 274–281
27. Nolan, G. P. (1994) Cell 77, 795–798
28. J. a. j., McCaffrey, P. F., Valge-Archer, V. E., and Rao, A. (1992) Nature 356, 801–804
29. McCaffrey, P. F., Luo, C., Kerpola, T., J. a. j., Bandalain, T., Ho, A. M., Burgon, E., Lane, W. S., Lambert, J. N., Curran, T., Verding, G. L., Rao, A., and Hogan, P. G. (1993) Science 262, 750–754
30. O’Reilly, D. R., Miller, K. L., and Luckow, V. A. (1992) Baculovirus Expression Vectors, Freeman and Company, New York
31. Ragona, G., Edwards, S. A., Mercola, D. A., Adamson, E. D., and Calogero, A. (1993) DNA Cell Biol. 10, 61–66
32. Chirgwin, J. M., Przybylo, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
33. Wright, J. J., Gunther, K., Mitsuya, H., Irving S. G., Kelly, K., and Siebenlist, U. (1990) Science 248, 588–591
34. Emmel, A. E., Verweij, C. L., Durand, D. B., Higgins, K. M., Lacy, E., and Crabbe, G. R. (1989) Science 246, 1617–1620
35. Dynan, D. W., and Tjian, R. (1993) Curr. Opin. Cell Biol. 5, 79–87
36. Jones, K. A., Kadowana, J. T., Liu, P. A., and Tjian, R. (1986) Science 232, 755–759
37. Nardelli, J., Gibson, T. J., Vesque, C., and Crabbe, P. (1991) Nature 349, 125–178
38. Yu, C.-Y., Mokam, K.-C., Chen, J., Baily, A. D., and Shen, C. K. (1991) J. Biol. Chem. 266, 8907–8915
39. Ackermann, S. L., Minden, A. G., Williams, G. T., Bonobon, C., and Yeung, C. Y. (1992) Proc. Natl. Acad. Sci. U. S. A. 88, 7523–7527
40. Shingu, T., and Bornstein, P. (1994) J. Biol. Chem. 269, 32551–32557
41. Gashler, A. L., Swaniwanathan, S., and Sukhatme, V. P. (1993) Mol. Cell. Biol. 13, 4536–4571
42. Verweij, C. L., Guidos, C., and Crabbe, G. R. (1990) J. Biol. Chem. 265, 15778–15795
43. Chen, D., and von Rothenburg, E. (1993) Mol. Cell. Biol. 13, 228–237
44. Flanagan, W. M., Corthesy, B., Bram, R. J., and Crabbe, G. R. (1991) Nature 352, 803–807

### Table 1

Luciferase activity of IL-2 promoter constructs in Jurkat T cells.

| Construct         | Light units | -Fold induction |
|-------------------|-------------|-----------------|
|                   | Uninduced   | Induced         |
| pCILuc1           | 421 (166)   | 40 630 (5821)   |
| pCILuc2           | 455 (165)   | 28 785 (3802)   |
| pCILuc3           | 479 (293)   | 10 502 (2000)   |
| pMILuc1           | 416 (117)   | 7 309 (1427)    |
| pMILuc2           | 190 (36)    | 851 (359)       |
| pMILuc3           | 438 (139)   | 1 417 (540)     |
| pMILuc4           | 165 (47)    | 341 (123)       |

### Fig. 7.

Conservation of ZIP, NFAT, and AP-1 sites in the human and mouse IL-2 promoter region. A, the nucleotide sequences of the human IL-2 promoter region, position –303 to –250 bp upstream the transcriptional start site is aligned with the mouse IL-2 promoter region. The ZIP, NFAT, and AP-1 sites are boxed, the region inserted in the mouse promoter is indicated by colons. B, alignment of the complementary G-rich zinc finger binding elements of the human and mouse IL-2 gene promoters.