Pleskot in 1974. Their spectrum of H_2S frost shows a very broad band centered near 3.9 μm.
22. We did this at the Jet Propulsion Laboratory, using a liquid N_2–cooled stainless steel platen in a vacuum environmental chamber coupled to an IR spectrometer with KBr windows. Frosts were grown in two ways: (i) as individual frost layers with H_2S deposited either directly on the horizontal platen or on top of a previously deposited SO_2 frost substrate; and (ii) as a two-component frost formed by the cocondensation of a mixture of SO_2 and H_2S gases. We measured the spectra using a Fourier-transform IR spectrometer [D. Nash, *Appl. Opt.* **25**, 2427 (1986)] with a resolution of 4 cm^{-1}; we measured the IR biconical diffuse reflectance of the frost deposits as they sublime and change composition as a result of differential sublimation of the two volatile species. These fine-grained, highly porous frost deposits, which visually appear bright white, were grown to various bulk thicknesses up to ~2 mm.
23. Probably either ν_1 or ν_3 or a near coincidence of the two. J. R. Ferraro and U. Fink, *J. Chem. Phys.* **67**, 409 (1977); J. R. Ferraro, G. Sill, U. Fink, *Appl. Spectrosc.* **34**, 525 (1980).
24. The errors in spectra B, C, and F of Fig. 2 are approximately 5%. However, spectrum F is the average of four successive independent measurements (taken over a 37-min period), which show general agreement, and therefore features in it can be considered reliable. Spectrum B and spectrum C result from a single sequence of measurements stepping through the wavelength interval. Undetected problems, such as clouds or miscentering, could result in a dip in the spectrum at the wavelength region where this problem occurred. However, no such problems were noticed at the time, but they cannot be completely excluded. Additional discussion of observational and data reduction procedures can be found in (*1*).
25. L. A. Lebofsky and M. B. Fegley, *Icarus* **28**, 379 (1976).
26. F. C. Witteborn, J. D. Bregman, J. B. Pollack, *Science* **203**, 643 (1979); J. Goguen *et al.*, *Icarus* **76**, 465 (1988); T. V. Johnson *et al.*, *Science* **242**, 1280 (1988).
27. D. P. Simonelli and J. Veverka, *Icarus* **74**, 240 (1988); A. S. McEwen, T. Johnson, D. Matson, L. Soderblom, *ibid.* **75**, 450 (1988). At temperatures of 120 to 140 K, the equilibrium vapor pressure of H_2S is about 10^{-5} to 10^{-3} atm [R. Honig and H. Hook, *RCA Rev.* (September 1960), p. 360].
28. D. Matson, T. Johnson, A. McEwen, L. Soderblom, *Bull. Am. Astron. Soc.* **20**, 1125 (1988) (abstract).
29. R. B. Minton, *Commun. Lunar Planet. Lab.* **10** (no. 188), 35 (1973).
30. R. Steudel, G. Holdt, A. Young, *J. Geophys. Res.* **91**, 4971 (1986).
31. J. Pearl, personal communication; F. Salama, S. Sandford, L. Allamandola, D. Cruikshank, F. Witteborn, *Bull. Am. Astron. Soc.* **20**, 818 (1988).
32. R. W. Carlson, D. Matson, T. Johnson, J. Bergstralh, *Astrophys. J.* **223**, 1082 (1978); W. H. Smyth and M. Combi, *ibid.* **328**, 888 (1988).
33. We thank J. Pearl, D. Matson and F. Salama for helpful comments and suggestions and J. Pearl, S. Baloga, and J. Goguen for reviewing the manuscript. This work benefited from discussions that took place at the Io Clutch held in San Juan Capistrano on 18 to 19 August 1988, attended by D. Matson, J. Pearl, J. Lunine, T. Johnson, A. McEwen, J. Moses, F. Fanale, R. Gaskell, S. Baloga, and the authors. Portions of this research were carried out at the Jet Propulsion Laboratory under National Aeronautics and Space Administration (NASA) contract and at San Juan Capistrano Research Institute under NASA grant NAGW-1350, both sponsored by the NASA Planetary Geology and Geophysics Program. Additional support was provided to the University of Wyoming through grant NAGW-1276 supported by the NASA Planetary Astronomy Program. This is San Juan Capistrano Research Institute contribution no. 1.

29 November 1988; accepted 24 February 1989

Kappa B–Specific DNA Binding Proteins: Role in the Regulation of Human Interleukin-2 Gene Expression

BEATRICE HOYOS, DEAN W. BALLARD, ERNST BÖHNLEIN, MIRIAM SIEKEVITZ, WARNER C. GREENE

Transcriptional activation of the human interleukin-2 (IL-2) gene, like induction of the IL-2 receptor α (IL-2Rα) gene and the type 1 human immunodeficiency virus (HIV-1), is shown to be modulated by a κB-like enhancer element. Mutation of a κB core sequence identified in the IL-2 promoter (−206 to −195) partially inhibits both mitogen- and HTLV-I Tax-mediated activation of this transcription unit and blocks the specific binding of two inducible cellular factors. These κB-specific proteins (80 to 90 and 50 to 55 kilodaltons) similarly interact with the functional κB enhancer present in the IL-2Rα promoter. These data suggest that these κB-specific proteins have a role in the coordinate regulation of this growth factor–growth factor receptor gene system that controls T cell proliferation.

THE GROWTH OF HUMAN T LYMPHOcytes is regulated in part by antigen- or mitogen-induced expression of the cellular genes encoding IL-2 and the α subunit of the high-affinity IL-2 receptor (IL-2Rα, Tac, and p55) (*1*). Similarly, the long terminal repeat (LTR) of the type 1 human immunodeficiency virus (HIV-1) is stimulated by T cell mitogens (*2–4*), which contributes to the heightened state of HIV-1 replication that occurs in activated $CD4^+$ T lymphocytes but not in resting cells (*5*). The induced expression of both the HIV-1 LTR (*2–4*) and the IL-2Rα promoter (*6–8*) in Jurkat T cells by mitogens such as phytohemagglutinin (PHA), phorbol 12-myristic 13-acetate (PMA), or the human T cell lymphotropic virus type I (HTLV-I)–derived Tax protein may involve the action of related κB enhancer elements. These enhancer motifs directly interact with at least two inducible cellular proteins including NF-κB (51 kD) (*9*) and HIVEN86A (86 kD) (*6, 7, 10*).

We now describe our studies on the role of identical or related factors in the coordinate activation of the human IL-2 gene. Inspection of the 5′ flanking region of the IL-2 gene (*11, 12*) revealed a 12-bp promoter sequence located between nucleotides −206 and −195 (AGGGATTTCACC) that resembled the κB enhancer elements present in the IL-2Rα promoter (GGGGAATCTCCC) and the HIV-1 LTR ($^{A}_{G}$GGGACTTTCC). Although several functional domains have been defined in the IL-2 promoter (*13, 14*), regulatory effects mediated through this putative κB site have not been described.

To examine whether this IL-2 κB-like element functioned in IL-2 promoter induction, we used oligonucleotide-directed mutagenesis to alter selectively various nucleotides within the putative κB site of the wild-type IL-2 promoter (*13*). One clustered mutation, designated M1 (GGG→CTC at −205 to −203), was specifically selected because identical base substitutions in the κB elements of the IL-2Rα promoter and HIV-1 enhancer reduce mitogen- or *tax*-induced promoter activation as well as κB-specific protein binding activity (*2, 6–8*). The additional M4 and M5 mutations altered four and eight bases within this site, respectively, while the M6 mutation changed three bases located immediately 5′ of the κB-like element. The IL-2 M1, M4, M5, and M6 mutant promoters and wild-type counterpart (*14*) were subsequently linked to the chloramphenicol acetyltransferase (CAT) gene for comparative transient expression studies. These recombinant reporter plasmids, either alone or in combination with sense or antisense *tax* expression vectors (spFMT2LTR 82-2C and 82-4, respectively) (*15*), were introduced into Jurkat T cells, that were then stimulated with PHA or PMA (Fig. 1). In accord with our earlier studies (*15*), combinations of PHA plus PMA, Tax plus PHA, and Tax plus PMA increased CAT activity in cells transfected with pIL-2-CAT, whereas single agents were largely ineffective. In contrast, introduction of the M1, M4, and M5 mutations in IL-2 promoter inhibited the response obtained with each of these combinations of

B. Hoyos and M. Siekevitz, Mount Sinai Medical Center, Department of Microbiology, New York, NY 10029. D. W. Ballard, E. Böhnlein, W. C. Greene, Howard Hughes Medical Institute, Departments of Medicine and Microbiology-Immunology, Duke University Medical Center, Durham, NC 27710.

stimuli. The M6 mutation located outside of the κB element, however, did not alter activation induced by Tax and PHA or PMA and only modestly affected the response induced by PHA and PMA combined. Together, these findings suggest that the IL-2 κB-like motif is needed for optimal mitogen-induced activation of the IL-2 promoter in these Jurkat cells.

To ascertain whether inducible cellular proteins specifically interact with this IL-2 κB-like site, we performed gel retardation assays with 22-bp oligonucleotides spanning this region. Electrophoretically retarded DNA-protein complexes were detected with nuclear extracts isolated from Jurkat T cells stimulated for 8 hours with PHA, PMA, or PHA plus PMA but not with media alone (Fig. 2A). These complexes of ^{32}P-labeled DNA and protein resulted from sequence-specific binding in that their formation was blocked by an excess (500 times, molar) of unlabeled wild-type IL-2 κB probe (Fig. 2B, lane 2). Consistent with the results of the functional assay (Fig. 1), size-matched IL-2 κB oligonucleotides that contained the M1 mutation failed to inhibit nucleoprotein complex formation (Fig. 2B, lane 3). Protein binding was also blocked by the addition of unlabeled oligonucleotides corresponding to the 5′ κB element of the HIV-1 enhancer (lane 4) or the κB element from the IL-2Rα gene (lane 6), but not by complete HIV-1 enhancer oligonucleotides containing the M1 mutation in both κB motifs (lane 5). Together, these cross-competition studies suggest that proteins interacting with the IL-2 κB site also bind to the HIV-1 enhancer and IL-2Rα κB elements but not to the M1 mutated version of these elements.

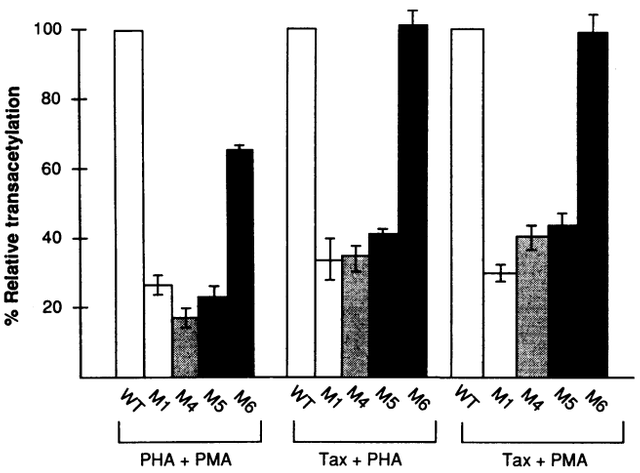

Fig. 1. A κB-like element in the IL-2 gene is required for optimal promoter activation mediated by combinations of PHA, PMA, and Tax. CAT expression vectors containing the wild-type IL-2 promoter (*13*) and wild-type κB sequence (AGGGATTTCAC, −206 to −195), or various mutations of this element (M1: ACTCATTTCAC; M4: AGGGAGGACAG; and M5: ACTCCGAACAG) or a three-base alteration immediately 5′ of the κB site (M6: AAG→TTC, −209 to −207) were cotransfected into Jurkat T cells in the presence of sense or antisense *tax* expression plasmids (*15*). The cells were stimulated 24 hours later with PHA (1 μg/ml), PMA (50 ng/ml), or combinations of these agents. CAT activity and total protein recovery in the transfected cultures were measured after an additional 24 hours of culture (*15*). Data for the mutants are presented as the percentage of the induced response of the wild-type IL-2 promoter (shown as 100% for each set of stimuli). The results with the M1 mutant reflect cumulative data from five experiments with five independent plasmid preparations, whereas the M4, M5, and M6 results are cumulated from three experiments performed with two independent plasmid preparations. Error bars indicate standard errors of the mean.

To compare quantitatively the binding of these inducible factors to the IL-2 κB element and to the HIV-1 enhancer (5′ motif), we performed gel-retardation assays with ^{32}P-labeled IL-2 κB and HIV-1 κB probes in the presence of graded amounts of unlabeled IL-2 and HIV-1 κB oligonucleotides (Fig. 2C). With IL-2 κB as the radioactive substrate (Fig. 2C, top), the IL-2 κB and HIV-1 κB competitor DNAs produced an essentially identical degree of inhibition at each concentration tested. In contrast, when HIV-1 κB was used as the ^{32}P-labeled probe (Fig. 2C, bottom), the IL-2 κB oligonucleotide consistently proved less effective as a competitor than the HIV-1 κB probe. The data suggest that these DNA binding proteins preferentially interact with the HIV-1 LTR κB element relative to the IL-2 κB site.

To examine whether the same inducible proteins interact with the κB elements of the IL-2 and IL-2Rα genes, we used bromodeoxyuridine-substituted, ^{32}P-labeled oligonucleotides (27 bp) containing these related, but nonidentical elements (Fig. 3) in ultraviolet light–induced cross-linking studies. Nuclear extracts from PMA-induced Jurkat T cells were incubated with these cross-linking probes in the presence or absence of unlabeled competitor oligonucleotides. After irradiation with ultraviolet light, we used preparative gel retentions to separate the nucleoprotein complexes from free DNA. Competition with the unlabeled wild-type IL-2Rα and IL-2 κB oligonucleotides (Fig. 3A, lanes 2 and 5) or those from which the κB site had been deleted (lanes 3 and 6) confirmed the κB-specific nature of the ^{32}P-labeled DNA-protein complexes formed. Subsequent analysis of these complexes on denaturing SDS–8% polyacrylamide gels revealed that proteins of identical size were covalently captured by both the IL-2 and IL-2Rα κB probes. These protein-DNA adducts included two species migrating with an apparent molecular size of 80 to 90 kD and two additional species at 50 to 55 kD (Fig. 3B). These findings suggested

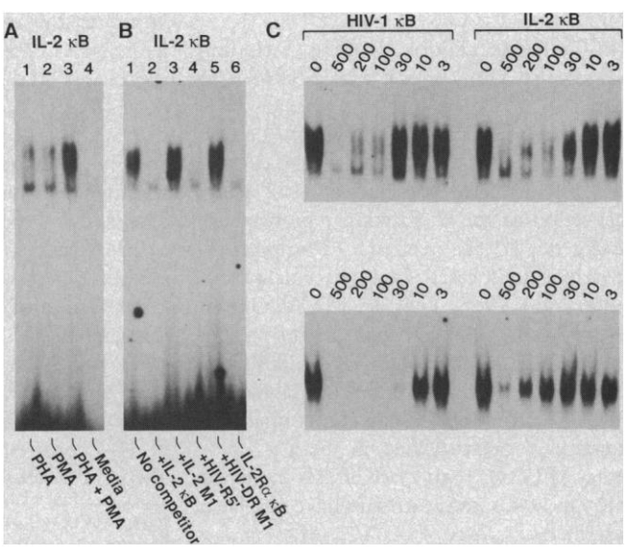

Fig. 2. Binding of inducible nuclear proteins to the IL-2 κB motif (**A**) Nuclear extracts (≃10 μg) from Jurkat T cells were prepared 8 hours after stimulation with PHA (2 μg/ml, lane 1), PMA (50 ng/ml, lane 2), or PHA plus PMA (lane 3), or in the absence of mitogen (lane 4), and mixed with ^{32}P-labeled IL-2 κB probe (*6*). Free DNA and protein-DNA complexes were separated by gel retardation (*6*) and detected by autoradiography. (**B**) Nuclear extracts from Jurkat T cells induced with PHA and PMA were prepared as in Fig. 2A and incubated with ^{32}P-labeled IL-2 κB oligonucleotides in the presence of an excess (500 times, molar) of the indicated unlabeled competitor oligonucleotides (*22*) as described (*6*). (**C**) Graded amounts of either unlabeled HIV-1 κB or IL-2 κB oligonucleotides were incubated with ^{32}P-labeled IL-2 κB (top) or HIV-1 κB (bottom) probes as described in (B). Only the retarded nucleoprotein complexes are shown.

that the same set of inducible T cell nuclear proteins specifically bind to the IL-2 κB and IL-2Rα κB elements. We obtained similar results with the HIV-1 κB elements except that lesser amounts of the 80 to 90 kD species were recovered.

Since the induction of NF-κB activity in B lymphocytes has been reported to be regulated at a posttranslational level (*16*) and to involve the induced dissociation of this factor from a labile cytoplasmic inhibitor (*17*), we next investigated the effects of cycloheximide on the mitogen-inducible expression of these T cell DNA binding proteins. As was expected, nuclear extracts from unstimulated Jurkat cells lacked binding activity (Fig. 4A, lane 1), whereas extracts from PHA- and PMA-induced cells contained proteins that formed complexes with the IL-2 κB probe (lane 2). Incubation of Jurkat T cells for 4.5 hours in 40 μ*M* cycloheximide, a concentration sufficient to inhibit 97% of de novo protein synthesis (*18*), induced small amounts of this DNA binding activity (lane 3). The addition of cycloheximide 30 min before stimulation with PHA and PMA (4 hours) did not alter the induction of this DNA binding activity (lane 4), a result consistent with posttranslational activation. Competition experiments with unlabeled derivatives of the IL-2 κB probe indicated that these mitogen-induced DNA binding proteins expressed in the cycloheximide-treated cells specifically interacted with the κB site (Fig. 4B). An excess (200 times, molar) of unlabeled wild-type IL-2 κB oligonucleotide or identical probes containing a three-base substitution (M3: CAA→GGG at −211 to −209) upstream of the κB element (−206 to −195) completely inhibited complex formation (Fig. 4B, lanes 2 and 6, respectively).

In contrast, unlabeled IL-2 κB probes containing either a 10-bp deletion in the κB element (IL-2 ΔκB) or three base pair substitutions within the κB element at −205 to −203 (M1: GGG→CTC) or −201 to −199 (M2: TTT→CCC) failed to compete for protein binding (lanes 3, 4, and 5, respectively). Protein binding to the IL-2 κB probe was also blocked by unlabeled oligonucleotides corresponding to the κB element and flanking region of the IL-2Rα gene (lane 7), the 5′ and 3′ reiterated elements of the HIV-1 enhancer (lanes 9 and 10), the κB element from the κ light chain immunoglobulin gene (lane 11) and the enhancer element from the murine class I major histocompatibility gene complex (H-2K κB, lane 12). However, an IL-2Rα oligonucleotide lacking the κB element did not compete (lane 8). Together, these results indicate that the κB elements from various viral and cellular genes are able to cross-compete for protein binding to the IL-2 κB element and that these proteins remain inducible in Jurkat T cells despite the inhibition of de novo protein synthesis.

We next investigated the question of whether these κB-specific proteins alone were sufficient to support IL-2 gene activation in Jurkat T cells. IL-2Rα and IL-2 mRNA expression was evaluated in Jurkat cells induced with PHA and PMA in the presence and absence of cycloheximide (Fig. 4C). Although IL-2Rα mRNA expression was well preserved in the cycloheximide-treated Jurkat T cells induced with PHA and PMA, IL-2 mRNA expression was undetectable. Similar results in Jurkat T cells have been reported by Shaw *et al.* (*14*) and Weiss *et al.* (*19*). Thus, although the κB-specific binding proteins are fully induced in the presence of cycloheximide, IL-2 gene activation does not occur. These findings suggest that the de novo synthesis of an additional

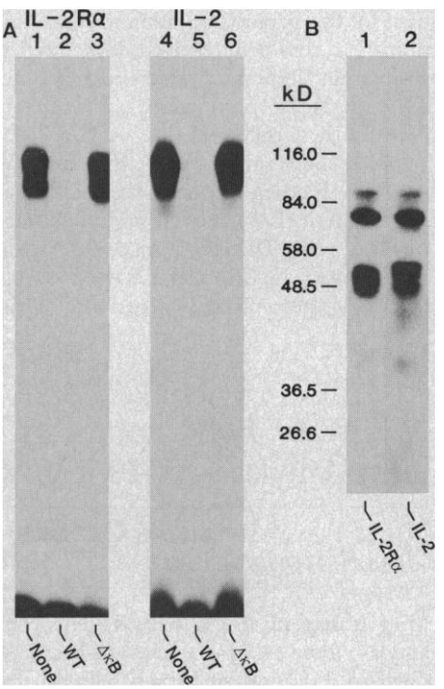

Fig. 3. The same nuclear factors directly bind to the κB elements present in the IL-2 and IL-2Rα promoters. (**A**) Nuclear extracts (≈20 μg) prepared from PMA-stimulated Jurkat T cells (*6*) were incubated with size-matched, bromodeoxyuridine-substituted, ^{32}P-labeled κB probes from either the IL-2Rα (nucleotides −275 to −249, lanes 1 to 3) or IL-2 (nucleotides −212 to −186, lanes 4 to 6) promoters in the absence (lane 1 and 4) or presence (lanes 2, 3, 5, and 6) of an excess (500 times, molar) of unlabeled competitor DNAs. Competitors included the homologous probe (lanes 2 and 4) or a κB-deleted variant (lanes 3 and 6) with fused 5′ and 3′ sequences flanking the corresponding κB motif. The reaction mixtures were irradiated with ultraviolet (UV) light for 30 min, and free and protein-complexed ^{32}P-labeled DNA species were separated by gel retardation (*6*). (**B**) Polyacrylamide slices containing adducts of the ^{32}P-labeled DNA and protein formed with ^{32}P-labeled IL-2Rα κB (lane 1) or ^{32}P-labeled IL-2 κB (lane 4) were excised from the retention gels (A) and analyzed directly on denaturing SDS–8% polyacrylamide gels. Molecular size markers are indicated.

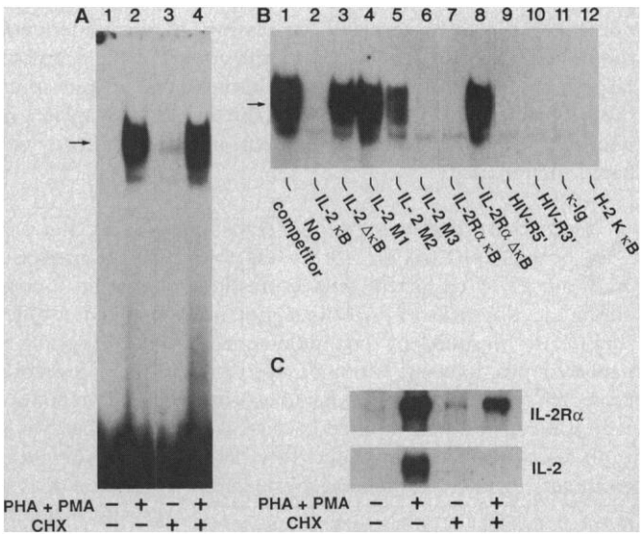

Fig. 4. DNA binding properties of nuclear extract proteins from Jurkat T cells treated with cycloheximide. (**A**) Effects of cycloheximide (CHX) on the induction of DNA binding proteins reactive with the IL-2 κB probe. Jurkat T cells were incubated with or without cycloheximide (40 μ*M*) for 30 min and then medium or combinations of PHA (1 μg/ml) and PMA (50 ng/ml) were added. Nuclear extracts were prepared 4 hours later and gel retardations performed with the ^{32}P-labeled IL-2 κB probe. Culture conditions for each extract are indicated at the bottom of the gel. (**B**) Specificity of proteins that are induced by PHA and PMA in the presence of cycloheximide that bind to the ^{32}P-labeled IL-2 κB probe. Gel retardation assays were performed with and without an excess (200 times, molar) of the indicated unlabeled oligonucleotides. Only the electrophoretically retarded complexes are presented. (**C**) RNA blot analysis of IL-2Rα and IL-2 mRNA expression in Jurkat T cells induced with PHA and PMA in the presence or absence of cycloheximide. The same Jurkat cell cultures described in (A) were used for the isolation of total cellular RNA. The upper and lower blots show expression of the 25*S* IL-2Rα mRNA and 11*S* IL-2 mRNA under the indicated conditions of induction.

factor (or factors) is required for IL-2 gene expression in these Jurkat T cells. Recently, an inducible nuclear factor of activated T cells, NFAT-1, was shown to be involved in the control of IL-2 gene activation in Jurkat T cells (*14*). Since NFAT-1 expression is dependent on de novo protein synthesis in Jurkat cells, it is a candidate for being at least one of these additional factors. We (*20*) and others (*21*) have found that IL-2 gene expression in mitogen-activated peripheral blood lymphocytes is independent of protein synthesis. Although the biochemical basis for this paradoxical result in Jurkat and primary T cells is unresolved, it may reflect biologically important differences in transcription factor production or action (or both) in these cell types.

Our findings support a role for κB-specific DNA binding proteins in the overall regulation of IL-2 gene activation in human Jurkat T cells. That these inducible factors also regulate IL-2Rα gene activation suggests that these proteins contribute to the coordinate induction of these genes, both of which are critically involved in the control of human T cell growth.

REFERENCES AND NOTES

1. W. C. Greene, W. J. Leonard, J. M. Depper, *Prog. Hematol.* **14**, 283 (1986); T. Tanighuchi *et al.*, *Immunol. Rev.* **92**, 121 (1986).
2. G. Nabel and D. Baltimore, *Nature* **326**, 711 (1987).
3. M. Siekevitz *et al.*, *Science* **238**, 1575 (1987).
4. J. D. Kaufman *et al.*, *Mol. Cell. Biol.* **7**, 3759 (1987); S. E. Tong-Starksen, P. A. Luciw, B. M. Peterlin, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6845 (1987).
5. S. Z. Salahuddin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5530 (1985); D. Zagury *et al.*, *Science* **231**, 850 (1986); J. S. McDougal *et al.*, *J. Immunol.* **135**, 3151 (1985); T. Folks *et al.*, *Science* **231**, 600 (1986).
6. E. Böhnlein *et al.*, *Cell* **53**, 827 (1988).
7. D. W. Ballard *et al.*, *Science* **241**, 1652 (1988).
8. K. Leung and G. Nabel, *Nature* **333**, 776 (1988); S. Ruben *et al.*, *Science* **241**, 89 (1988).
9. K. Kawakami, C. Scheidereit, R. G. Roeder, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4700 (1988).
10. R. B. Franza *et al.*, *Nature* **330**, 391 (1987).
11. T. Fujita *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7437 (1983).
12. U. Siebenlist *et al.*, *Mol. Cell Biol.* **6**, 3042 (1986).
13. D. Durand *et al.*, *ibid.* **8**, 1715 (1988); G. Nabel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2934 (1988); T. Fujita *et al.*, *Cell* **46**, 401 (1986); T. M. Williams *et al.*, *J. Immunol.* **141**, 662 (1988); M. W. Brunvand *et al.*, *J. Biol. Chem.* **263**, 18904 (1988).
14. J.-P. Shaw *et al.*, *Science* **241**, 202 (1988).
15. M. Siekevitz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5389 (1987).
16. R. Sen and D. Baltimore, *Cell* **47**, 921 (1986).
17. P. A. Baeuerle and D. Baltimore, *ibid.* **53**, 211 (1988); *Science* **242**, 540 (1988).
18. D. W. Ballard and W. C. Greene, unpublished data.
19. A. Weiss *et al.*, *J. Immunol.* **138**, 2169 (1987).
20. M. Krönke *et al.*, *J. Exp. Med.* **161**, 1593 (1985).
21. S. Efrat and R. Kaempfer, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2601 (1984); A. Granelli-Piperino *et al.*, *J. Exp. Med.* **163**, 922 (1986).
22. The sequences of the oligonucleotides are: IL-2 κB, 5′ gatccAAAGAGGGATTTCACCT; IL-2 M1, 5′ gatccAAAGACTCATTTCACCT; HIV-R5′, 5′ gatccAGGGACTTTCC; HIV-DR M1, 5′ ACAACTCACTTTCCGCTGCTCACTTTCCAGGG; IL-2RκB, 5′ gatccGGCAGGGAATCTCCCT; where gatcc represents nonspecific linker sequence added to facilitate cloning.

23. We thank H. Bogerd, J. Hoffman, and D. Shurland for technical assistance and N. Holbrook and G. Crabtree for the pIL-2-CAT plasmid. Supported in part by grants to M.S. from the National Institute of Health (A127053-01) and the Life and Health Insurance Medical Research Fund.

9 November 1988; accepted 28 February 1989

Metastatic Hibernomas in Transgenic Mice Expressing an α-Amylase–SV40 T Antigen Hybrid Gene

NILES FOX,* ROSANNE CROOKE, LIH-HWA S. HWANG,† UELI SCHIBLER, BARBARA B. KNOWLES, DAVOR SOLTER

Mice transgenic for a hybrid gene containing the liver promoter of the mouse α-amylase gene (*Amy-1*^{a}) fused to the SV40 tumor antigen coding region unexpectedly developed malignant brown adipose tissue tumors (malignant hibernomas). Expression of the α-amylase gene had previously been thought to be confined to the liver, parotid, and pancreas; however, analysis of white and brown adipose tissue from nontransgenic mice revealed expression of the endogenous *Amy-1*^{a} gene in these tissues. Gene constructs driven by the *Amy-1*^{a} liver promoter thus provide a means of targeting gene expression to the adipocyte cell lineage in transgenic mice. Moreover, the high frequency of metastases in the liver, lungs, spleen, heart, and adrenals of these mice provides an experimental system in which to study the development of disseminated malignancy.

STUDY OF THE MOUSE α-AMYLASE gene *Amy-1*^{a} has provided a valuable model of regulated gene expression during development (*1*). Transcription of *Amy-1*^{a} is controlled by two differentially regulated promoters; a stronger upstream promoter (the parotid promoter) regulates expression in the parotid gland, whereas a weaker downstream promoter (the liver promoter) regulates expression in the liver, parotid, and pancreas. Each promoter controls transcription of an mRNA containing a specific nontranslated leader sequence that is joined by differential splicing to the shared *Amy-1*^{a} coding region. Activation of the two *Amy-1*^{a} promoters is temporally regulated in the developing parotid gland. Expression from the liver promoter is detectable at birth, whereas expression from the parotid promoter does not occur until 14 days postpartum. Adult levels of *Amy-1*^{a} mRNA are not attained until approximately 3 weeks after birth owing to the late activation of the parotid promoter (*1*). Little is known about the mechanisms regulating the tissue-specific expression of the *Amy-1*^{a} gene. Some information has been obtained from analysis of hybrid *Amy-1*^{a} gene constructs transfected into cells in culture and by in situ analysis of *Amy-1*^{a} mRNA expression in the mouse (*1*). However, these studies do not address the issue of which regions of flanking sequences regulate *Amy-1*^{a} expression within the various tissues of the organism. To study the regulation of this gene, we have produced transgenic mice harboring a hybrid gene consisting of the *Amy-1*^{a} liver promoter and the sequence encoding SV40 T antigen (Tag).

An Eco RI restriction fragment of the *Amy-1*^{a} gene was first isolated that contained 438 bp of the liver promoter and flanking region and 162 bp of the liver untranslated region (*2*). This fragment was then subcloned in front of the Hind III–Bam HI fragment encoding SV40 Tag (excluding the 21- and 72-bp repeats) in pBR322 Δ. The resulting plasmid, p600T, was linearized at the Bam HI site and microinjected into the pronuclei of fertilized one-cell stage C57B1/6J mouse embryos (*3*). Injected eggs were transferred into the oviducts of pseudopregnant outbred CD-1 mice and the mice that were born were analyzed for the presence of the transgene by Southern blotting. Seven transgenic mice containing between five and ten integrated copies of the p600T transgene were obtained and stable transgenic lineages were successfully developed from six of these by backcrossing to C57B1/6J mice and then crossing them among themselves (Table 1). One transgenic mouse that developed bilateral subscapular tumors was killed before a lineage could be established.

N. Fox, R. Crooke, L-H. S. Hwang, B. B. Knowles, D. Solter, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104.
U. Schibler, Department of Molecular Biology, University of Geneva, 30 Quai Ernest-Ansermet, CH-1211, Geneva 4, Switzerland.

*Present address: Eli Lilly, Lilly Research Laboratories, Lilly Corp. Center, Indianapolis, Indiana 46285.
†Present address: Development Center for Biotechnology, 81 Chang-Hsing Street, Taipei, Taiwan, ROC.