Importance of Low Affinity Elf-1 Sites in the Regulation of Lymphoid-specific Inducible Gene Expression

By Susan John,* Richard Marais,† Ryan Child,* Yvonne Light,‡ and Warren J. Leonard*

From the *Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892; †Cancer Research Campaign Center for Cell and Molecular Biology at the Institute of Cancer Research, Chester Beatty Laboratories, London SW3 6JB, United Kingdom

Summary

Elf-1 is an Ets family transcription factor that regulates a number of inducible lymphoid-specific genes, including those encoding interleukin 3 (IL-3), granulocyte/macrophage colony-stimulating factor (GM-CSF), and the IL-2 receptor (IL-2R) α chain. A minimal oligonucleotide spanning the IL-2Rα Elf-1 site (−97/−84) bound Elf-1 poorly, but binding activity markedly increased when this oligonucleotide was multimerized or flanking sequences were added. This result is consistent with the requirement of accessory proteins for efficient Elf-1 binding, as has been demonstrated for the GM-CSF and IL-3 promoters. A binding site selection analysis revealed the optimal Elf-1 consensus motif to be A(A/t)(C/a)CCGGAAGT(A/S), which is similar to the consensus motif for the related Drosophila E74 protein. This minimal high affinity site could bind Elf-1 and functioned as a stronger transcription element than the −97/−84 IL-2Rα oligonucleotide when cloned upstream of a heterologous promoter. In contrast, in the context of the IL-2Rα promoter, conversion of the naturally occurring low affinity Elf-1 site to an optimal site decreased inducible activation of a reporter construct in Jurkat cells. This finding may be explained by the observation that another Ets family protein, ERGβ/Fli-1, can efficiently bind only to the optimal site, and in this context, interferes with Elf-1 binding. Therefore, high affinity Elf-1 sites may lack sufficient binding specificity, whereas naturally occurring low affinity sites presumably favor the association of Elf-1 in the context of accessory proteins. These findings offer an explanation for the lack of optimal sites in any of the known Elf-1-regulated genes.

The Ets gene family encompasses a variety of DNA binding proteins involved in cellular growth and differentiation. Aberrant expression of some members of this family have been implicated in cellular transformation (for reviews see references 1–3). All Ets family proteins contain a conserved DNA binding “Ets” domain comprising adjacent α-helical and basic domains that is referred to as the Ets domain. The location of the Ets domain varies within members of this family, but regardless of location the Ets domain mediates binding to the core nucleotide sequence GGAA/T (2). Ets family proteins can be classified based on the structures of their Ets domains, with each subgroup displaying subtle differences in DNA binding specificity (4). The binding and transactivation of some Ets family proteins have been shown to be influenced by the binding of accessory proteins (5–13). Whereas some Ets family of proteins are ubiquitously expressed, others exhibit cell type-specific expression.

Elf-1 is an Ets family member whose expression is relatively restricted to lymphoid and myeloid cells (14). Although Elf-1 was identified by low stringency hybridization using a probe from the basic domain of human Ets-1, its Ets domain is most similar to that of the Drosophila E74 Ets family protein (14). Elf-1 can bind the underphosphorylated form of the retinoblastoma gene product (Rb) (15), so that during T cell activation, the phosphorylation of Rb results in the release of Elf-1, temporally correlating with the expression of Elf-1 regulated genes. Binding sites for Elf-1 have been identified in the promoters of a variety of important lymphoid-specific genes including those encoding IL-3 (5), GM-CSF (6), CD4 (16), and IL-2Rα (17), as well as in the long terminal repeats of the T cell tropic viruses HIV-2 (18) and human T cell lymphotropic virus I (HTLV-I) (19).

We have demonstrated that the IL-2Rα Elf-1 site is essential for transcriptional activation of this gene and established IL-2Rα as the first example of a gene in which an enhancer (positive regulatory region II; 17) can be activated by overexpression of Elf-1 and an accessory protein, the high mobility group protein (HMG-I) (17). It is interesting to note that Elf-1 exhibited essentially no binding to an oligonucleotide (−97 to −84 of the IL-2Rα promoter).
spanning only the core GGAA Ets recognition motif and flanking nucleotides identified by methylation and ethylation interference analyses. However, it avidly bound a larger fragment, indicating that Elf-1 binding was dependent on additional sequences and/or accessory proteins. A binding site selection analysis defined a 12-nucleotide consensus motif spanning a GGAA core motif as the optimal Elf-1 site. It is interesting to note that the −97/−84 IL-2Rα oligonucleotide lacks two of the highly conserved nucleotides, explaining its poor Elf-1 binding activity. Conversion of the naturally occurring, lower affinity Elf-1 site in the IL-2Rα promoter to an optimal site resulted in a decrease in inducible transcriptional activity. We show that the ets family protein, ERGB/Fli-1, can bind only to the high affinity site and can efficiently interfere with Elf-1 binding in this context. Therefore, high affinity Elf-1 sites may lack sufficient binding specificity to allow carefully controlled Elf-1-mediated gene regulation. These studies suggest that the presence of naturally occurring high affinity Elf-1 sites in Elf-1 regulated genes could be a mechanism to achieve greater inducibility and underscore the importance of accessory proteins in modulating Elf-1 binding and action.

Materials and Methods

Electrophoretic Mobility Shift Assays and Methylation and Diethylypyrocarbonate Interference Assays. Binding reactions (final volume, 20 µl) contained 2 µl of in vitro translated proteins or 3 µl of a 1:10 dilution of SF9 insect cell extracts (control or Elf-1 programmed), 10,000–40,000 cpm of probe (0.1–0.3 ng), 2 µg of Elf-1 sites in Elf-1 regulated genes could be a mechanism that suggests the absence of naturally occurring high affinity binding sites in Elf-1 regulated genes. These studies controlled Elf-1-mediated gene regulation. These studies suggest that the presence of naturally occurring high affinity Elf-1 sites in Elf-1 regulated genes could be a mechanism to achieve greater inducibility and underscore the importance of accessory proteins in modulating Elf-1 binding and action.

Materials and Methods

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Oligonucleotides, Plasmids, and In Vitro Mutagenesis. Oligonucleotides were synthesized on a DNA/RNA synthesizer (model 392; Applied Biosystems, Inc., Foster City, CA). The IL-2Rα −97 to −74 (5′-agcttCACTTCCTATATTT-3′ top strand; 5′-AAATATGAGAAGATGAGG-3′ bottom strand) and 3× (−97/−84) (5′-agcttCACTTCCTATATTTGAGATGAGAAGTG-3′) oligonucleotides were synthesized with HindIII and BamHI overhangs. The oligonucleotides depicted (see Figs. 3 A and 4 A) had HindIII- and XbaI-compatible ends and were cloned between these sites in pBLCAT2. The mutant IL-2Rα construct (IL-2Rα m2) was generated by PCR mutagenesis (20). The sequence on the top strand of the wild-type (WT) and mutant −137/−64 m2 oligonucleotides used in EMSAs are as follows: −137/−64 WT 5′-agcttAGCCAACACTATATGCTAC(A)5′-AGCCAAGAGAAGAGAGG-3′ and −137/−64 m2 5′-agcttAGCCAACACTATATGCTAC(A)5′-AGCCAAGAGAAGAGAGG-3′

Binding Site Selection. A Myc epitope tag (21) was inserted into the Elf-1 cDNA between amino acids 5 and 6 by PCR mutagenesis (20); this Myc-tagged Elf-1 ("Elf-tag") construct was cloned into pcDNA I/Amp (Invitrogen, San Diego, CA) between the EcoRV and XhoI sites, and correctness was verified by DNA sequencing. A binding site selection assay (22) was performed using a pool of oligonucleotides containing a central core of 26 random nucleotides. After the binding of in vitro translated Elf-tag protein, sequences that could bind Elf-1 were immunoprecipitated with 9E10, an anti-Myc mAb (21), and amplified by PCR. After four rounds of selection, DNA was gel purified and analyzed by DNA sequencing.

Expression and Purification of Elf-1 In Insect Cells. We first constructed a versatile baculovirus expression vector, pVLHSPlink, that contains a polylinker to facilitate cloning of a cDNA insert. When expressed in SF9 cells, pVLHSPlink directs production of the cDNA-encoded peptide as a fusion protein containing a run of six histidines at the NH2 terminus (R. Marais, manuscript in preparation). The 1.9-kb Ncol to Xbal human Elf-1 cDNA fragment from pEF-Elf-1plink (S. John, unpublished observations) was inserted between the Ncol and Xbal sites of pVLHSPlink. The resulting plasmid, pVLHSElf-1, was used to prepare an insect cell virus, denoted H2 Elf-1, using the Baculo-Gold kit (PharMingen, San Diego, CA) as a source of linearized viral genomic DNA. The expressed His6-Elf-1 fusion protein was purified using Ni2+ affinity chromatography. To generate a control virus, we used pVLHASP-AS-SAP-1, in which the Ets family protein SAP-1 (13) was cloned in an antisense orientation (R. Marais, unpublished observations). SF9 insect cells were propagated and viral infection performed as previously described (23), except that the cells were grown in 8s 900-II defined medium supplemented with antibiotics. Cell extracts were prepared as described (23).

Cell Lines, Transfections, and CAT Assays. Jurkat E6.1 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Biofluids, Inc., Rockville, MD), penicillin (100 U/ml), streptomycin (100 U/ml), and glutamine (2 mM). Transfections were performed by the DEAE-dextran method (17). After transfection, cells were maintained in 2% fetal bovine serum and then were either left untreated or treated with PMA (20 ng/ml; Calbiochem-Novabiochem Corp., La Jolla, CA) for 12–15 h. CAT assays were performed as described (17) using equal amounts of protein in each assay.

Results and Discussion

Identification of a Consensus Motif for Optimal Elf-1 Binding. We have demonstrated that Elf-1 regulates transcription of the IL-2Rα gene and contacts the −97 to −84 sequence (5′-CACTTCCTATATT-3′ top strand; 5′-AAATATGAGAAGATGAGG-3′ bottom strand; GGAA Ets core motif underlined) in the IL-2Rα promoter in positive regulatory region II (PBR II) (17). It is interesting to note that an oligonucleotide comprising only this region (Fig. 1 A, lane 2) bound Elf-1 weakly, whereas a trimer of this region (lane 4) or a longer oligonucleotide (−97 to −74, lane 6) exhibited greater Elf-1 binding. To clarify Elf-1 binding nucleotide preferences, we used a binding site selection method (22). As expected, no complex was detected when the initial random pool of oligonucleotides was used as a probe (Fig. 1 B, lane 1), but after two or more rounds of selection, two specific complexes were formed with the Elf-1 cDNA programmed lysate (lanes 3–5) but not with unprogrammed lysate (lane 6). The slower mobility complex
comigrated with the complex formed with nuclear extracts, whereas the faster mobility complex appears to result from degradation of Elf-1 (17). DNA from each complex was cloned into pBluescript. Of the 50 clones derived from the slower mobility complex, 49 contained a GGAA motif, with one of these having two GGAA motifs (Table 1).

Alignment of the 48 sequences containing a single motif (Table 1, Consensus) revealed C residues were present in 98 and 94%, respectively, of the sequences at the -2 and -1 positions, and a preference for a C at the -3 position and A at the -4 and -5 positions. Downstream of the GGAA motif, a G at position +1, a T at position +2, and an A, G, or C at position +3 were preferred. Thus, the consensus motif for optimal Elf-1 binding was 5' AA/tC/aCCGGAAGTa/g/c-3'.

To confirm that the selected sequences could bind Elf-1, probes that encompassed much of the sequence variation of the selected clones (CCGGAA [nos. 20 and 85], CAGGAA [no. 78], and ACGGAAGT [no. 53] core sequences; Table 1) were tested in EMSAs using extracts from insect cells infected with a control empty virus or with a virus encoding Elf-1 containing six histidines at its NH2 terminus (H6Elf-1, Fig. 1 C). H6Elf-1 formed specific complexes with all four probes but not with a probe derived from clone 21 (data not shown), which lacks an Ets binding motif (Table 1) and whose selection probably resulted from nonspecific binding. Similar results were obtained with in vitro translated Elf-1, whereas unprogrammed lysate did not form specific complexes (data not shown). Clone 85 exhibited the highest binding activity for H6Elf-1 (Fig. 1 C, lane 8), consistent with this clone containing the preferred nucleotides at all positions (Table 1). Clones 78, 53, and 20 (which contain A’s at the -1, -2, and +2 positions, respectively) each exhibited less binding than did clone 85 (lanes 5–7 vs. 8), suggesting that C’s at -1 and -2 and a T at the +2 position are required for optimal Elf-1 binding. In addition to the Elf-1-specific complex formed with probe 53, two faster mobility complexes were observed (lane 6), but these were also formed with the control mock-infected extracts (lane 2) and therefore were not Elf-1 specific.

Identification of the Nucleotides Contacted by Elf-1 in the Consensus Motif. To determine which nucleotides were contacted by Elf-1, we performed methylation and carboxyethylination interference analyses using clone 85, which contains the high affinity Elf-1 binding sequence, 5’-AAC-CCGGAAGT-3’.

Methylation interference analysis revealed strong protection of the GGAA core on the top strand and the A at the +2 position on the bottom strand.
Table 1. Selected Binding Sites for Elf-1

| Site | Sequence                        |
|------|---------------------------------|
| 1    | CCCTTGTTCAAT                      |
| 2    | TCTCTCTGTTCAAT                    |
| 3    | ACCTCTCTGTTCAAT                   |
| 4    | GGTCTCTGTTCAAT                    |
| 5    | ATCTCTCTGTTCAAT                   |
| 6    | GCCTCTCTGTTCAAT                   |
| 7    | AGCTCTCTGTTCAAT                   |
| 8    | CGCTCTCTGTTCAAT                   |
| 9    | TTCTCTCTGTTCAAT                   |
| 10   | GTCTCTCTGTTCAAT                   |
| 11   | ATCTCTCTGTTCAAT                   |
| 12   | GCCTCTCTGTTCAAT                   |
| 13   | AGCTCTCTGTTCAAT                   |
| 14   | CGCTCTCTGTTCAAT                   |
| 15   | TTCTCTCTGTTCAAT                   |
| 16   | GTCTCTCTGTTCAAT                   |
| 17   | ATCTCTCTGTTCAAT                   |
| 18   | GCCTCTCTGTTCAAT                   |
| 19   | AGCTCTCTGTTCAAT                   |
| 20   | CGCTCTCTGTTCAAT                   |
| 21   | TTCTCTCTGTTCAAT                   |
| 22   | GTCTCTCTGTTCAAT                   |
| 23   | ATCTCTCTGTTCAAT                   |
| 24   | GCCTCTCTGTTCAAT                   |
| 25   | AGCTCTCTGTTCAAT                   |
| 26   | CGCTCTCTGTTCAAT                   |

The sequences of the selected oligonucleotides are depicted with the conserved Ets motif GGAA (boxed). The Elf-1 consensus motif (bottom) was derived from 48 sequences containing a single GGAA binding core; the frequency of each nucleotide used to generate the consensus is shown. At positions -4 and -3, the more favored nucleotide is depicted in uppercase and the second most favored residue in lowercase. At position +3, A, G, and C residues were selected at approximately the same levels and are therefore represented as a/s, where s = G or C. All sequences shown correspond to the strand containing the GGAA core motif.
Table 2. Known Elf-1 Binding Sites (Top) and Comparison of Consensus Binding Sites for Elf-1, E74, Fli-1, Ets-1, and Elk-1 (Bottom)

| Site                      | Percent identity to consensus Elf-1 site |
|---------------------------|-----------------------------------------|
| IL-2Rα*                   | ~78                                     |
| CD4                       | ~76                                     |
| GM-CSF                    | ~75                                     |
| IL-3                      | ~74                                     |
| HTLV-I LTR (Pub1)         | ~70                                     |
| HTLV-I LTR (Pub2)         | ~70                                     |
| HIV-2 LTR (Pub1)          | ~70                                     |
| HIV-2 LTR (Pub2)          | ~70                                     |

A A A C C C GG AGT A a, Elf-1*  
A A Y CM GG AG T E74  
N R A C C C AG T, Fli-1  
R CM GG AW R Y Ets-1  
a, C A, C a, C a, GG WT R Elk-1

(Top) IL-2Rα* refers to the −97 to −84 region of the IL-2Rα promoter (17); the noncoding strand is shown so that the orientation corresponds to the consensus shown in Table 1. The other Elf-1 sites are in the IL-3, GM-CSF, and CD4 enhancers, (5, 6, 16) and in the HIV-2 (18) and HTLV-I (19) LTRs. Also shown are the percent identity of the known Elf-1 sites with the 12-nucleotide Elf-1 consensus shown in Table 1. (Bottom) The consensus binding sites for optimal binding of Elf-1 is compared with that of Ets-1 (24, 25), E74 (26), and Fli-1 (27), and Elk-1 (30) are shown. The core Ets binding motif, GGAA/T is indicated in bold typeface.

Elf-1 site (Table 2). To clarify the basis for the lack of Elf-1 binding to the minimal −97 to −84 IL-2Rα oligonucleotide, we assayed the ability of Elf-1 to bind to a series of mutant oligonucleotides (Fig. 3 A). Analogous to in vitro translated Elf-1 (Fig. 1 A), purified recombinant His-tagged Elf-1 protein did not bind to the −97/−84 oligonucleotide (Fig. 3, B, lane 1), which lacks the consensus C at positions −1 and −2. Maximum Elf-1 binding activity was observed with m2, which contained C’s at these positions (lane 3). Mutant m1, which has a C at −2, bound Elf-1 weakly, but no significant binding activity was seen with m3, which contained a C at −1 (lanes 2 and 4), confirming that C’s at both the −1 and −2 positions are essential for optimal Elf-1 binding. The highly conserved A at −5 was also important, since its mutation to a G in the context of the high affinity m2 oligonucleotide (Fig. 3 A, m4), resulted in a large decrease in Elf-1 binding (Fig. 3 B, lanes 5 vs. 3). No binding to any probe was seen when control lysates were used (lanes 6–10). Therefore, the inability of Elf-1 to bind to the minimal −97 to −84 IL-2Rα oligonucleotide was due to the absence of the consensus C at positions −1 and −2.

High Affinity Elf-1 Sites Are Less Inducible than Suboptimal Sites. We next compared the ability of IL-2Rα −97/−84 oligonucleotides to activate transcription when cloned upstream of the TK promoter in pBLCAT2 and transfected into Jurkat T cells. The optimal Elf-1 site (m2 mutant) was a stronger transcriptional element than the WT −97/−84 IL-2Rα suboptimal Elf-1 binding site (Fig. 3 C), suggesting that the extent of activation in vivo correlates with the level of Elf-1 binding in vitro. Mutants m1 and m3 (Fig. 3 A) were transcriptionally less active than m2 (data not shown). We next studied the ability of the high affinity Elf-1 site to function in the context of the −472 to +109 full-length IL-2Rα promoter by transfecting WT and mutant IL-2Rα constructs into Jurkat T cells and assaying for transcriptional activity after PMA stimulation. In contrast to our finding with the minimal −97 to −84 construct, in the full-length promoter setting, conversion of the natural WT Elf-1 site to a high affinity Elf-1 (m2) site did not increase the basal promoter activity and was less inducible than the WT promoter (Fig. 3 D). The reason for the lower inducibility of the m2 mutant is likely due to its higher basal level of Elf-1 binding observed in EMSAs using Jurkat nuclear extracts (Fig. 3 E). An anti-Elf-1 antibody was able to supershift the upper complex formed with both WT and mutant oligonucleotides (Fig. 3 E).

ERG/Fli-1 Bind Efficiently to a High Affinity Elf-1 Site and Can Inhibit Elf-1 Binding. A comparison of the consensus sites for Elf-1, Ets-1 (5’-RCMGGAWRY-3’; references 24 and 25), E74 (5’-AAYCMGGAAGT-3’; 26), and Fli-1 (5’-NG/AACCGGAAG/A/T/cA/G-3’; 27) (see Table 2) revealed that as expected, the binding specificities for Elf-1 and E74 sites are more similar to each other than to the Ets-1 site. It is surprising that the optimal Elf-1 site is very similar to that of another Ets family protein, ERG, or its murine homologue, Fli-1 (both contain CCGGA core sequences; see Table 2), even though these two proteins belong to separate Ets protein subfamilies and contain only 46% amino acid identity in their Ets domains. Upstream of their GGAA cores there were more differences between the Elf-1 and ERG/Fli-1 binding sites, particularly at the −5 position where there is an absolute requirement for an A for optimal Elf-1 binding (Fig. 3 B, mutant m4), whereas Fli-1 can accommodate any nucleotide at this position (27). We therefore hypothesized that one of the reasons for the lower inducibility of the IL-2Rα m2 promoter than of the WT IL-2Rα promoter (Fig. 3 D) may be due to the ability of ERG/Fli-1 to also bind to the high affinity Elf-1 site, preventing Elf-1-mediated regulation of transcription. Indeed, whereas Elf-1 could bind to both WT and m2 −137/−64 oligonucleotides, in vitro translated ERG/Fli-1 only bound to the m2 oligonucleotide (Fig. 4 A). We confirmed that ERG/Fli-1 from nuclear extracts was also able to bind to the high affinity Elf-1 site by performing DNA affinity purification using Jurkat nuclear extracts and a biotinylated 3X (−97/−84 m2) oligonucleotide, followed by Western blot analysis using an anti-Fli-1 antibody (data not shown). Finally, we investigated whether Elf-1 and ERG/Fli-1 can compete with...
Figure 3. Elf-1 binding to WT and mutant IL-2Rα oligonucleotides. (A) Sequences of the −97/−84 IL-2Rα oligonucleotides used in B. The antisense strand of each oligonucleotide is shown. For m1, m2, m3, and m4, the mutated nucleotides are underlined. (B) Oligonucleotides were end-labeled and diluted to similar specific activity and evaluated in EMSAs using purified recombinant His6-tagged Elf-1 protein that was produced in Sf9 insect cells (lanes 1–5) or control Sf9 cell lysates (lanes 6–10). The probes are indicated above each lane. The position of the Elf-1 specific complex is indicated. (C) Jurkat cells were transfected with the control vector (pBLCAT2), or constructs containing the WT or m2 −97/−84 IL-2Rα oligonucleotides cloned upstream of TK-CAT in pBLCAT2. A representative experiment is shown. Relative CAT activities from three independent experiments normalized to the activity of pBLCAT2 in the absence of PMA, which was assigned a value of 1.0, were as follows: for uninduced cells pBLCAT2 (1, 1, 1); −97/−84TKCAT (3.4, 1.9, 2.7); m2TKCAT (4.8, 5.8, 3.6); for induced cells, pBLCAT2 (1.2, 1.4, 1.2), −97/−84TKCAT (4.9, 2.7, 3.8), m2TKCAT (7.7, 9.1, 8.1). (D) A high affinity Elf-1 site confers lower PMA-induced activity to the IL-2Rα promoter. Sequence of the −97/−84 region of the WT and mutant (m2) IL-2Rα promoter. The mutated nucleotides are underlined in IL-2Rα-m2. Jurkat E6.1 cells were transfected with control vector Jymcat-0 (J0) or with WT or m2 IL-2Rα constructs. Data represent the mean ± standard error of the mean of three (for J0) or four (for WT or IL-2Rα m2) independent experiments. The activity of J0 without PMA treatment was assigned a value of 1.0; the activities of other constructs are expressed as relative fold increases over J0 activity. (Open bar) No PMA treatment; (solid bar) PMA treated. (E) EMSAs were performed with 5 μg of nuclear extracts prepared from PMA-activated Jurkat cells that were incubated with no antibody (lanes 1 and 3) or 1 μl of an anti-Elf-1 mAb (17) (lanes 2 and 4) before incubation with 32P-labeled WT (lanes 1 and 2) or m2 (lanes 3 and 4) −137/−64 IL-2Rα oligonucleotides. (Arrow) Supershifted Elf-1 complex.

Each other for binding by performance EMSAs with a fixed amount of Elf-1 and increasing amounts of ERGB/Fli-1 (Fig. 4 B, lanes 2–6) or a constant amount of ERGB/Fli-1 and increasing amounts of Elf-1 (lanes 8–12). The binding of Elf-1 or ERGB/Fli-1 alone are shown in lanes 1 and 7, respectively. Whereas both Elf-1 and ERGB/Fli-1 could compete with each other for binding, ERGB/Fli-1 was more effective at displacing Elf-1, suggesting that it could bind with higher affinity to the −137/−64 m2 site than Elf-1.

In Jurkat T cells, ERGB/Fli-1 is constitutively expressed (our unpublished observations). Thus, the lower inducibility observed in Jurkat cells when the normal Elf-1 site in the IL-2Rα promoter was replaced with a high affinity Elf-1 site could be due to impaired regulation of the promoter resulting from competition between Elf-1 and ERGB/Fli-1 for the same site. Analogously, we have found that mutation of the GM-CSF PB1 enhancer (6) so that it now contains a high affinity Elf-1 site, results in a loss of the requirement for a cooperating AP-1 site for its functional activity, but displays reduced levels of inducibility when compared to its normal counterpart (data not shown). These studies suggest that the loss of fine DNA binding specificity associated with a high affinity site, could cause serious dysregulation of gene expression in a normal T cell environment, where a number of expressed Ets family proteins could potentially bind to the high affinity site.

Whereas Ets-1 and Ets-2 can utilize a GGAA or GGAT
ERGβ/Fli-1 can compete with Elf-1 for binding to the high affinity m2 Elf-1 site but cannot bind the WT IL-2Rα Elf-1 site. (A) EMSAs were performed with unprogrammed (lanes 1 and 4), ERGβ (lanes 2 and 5) or Elf-1 (lanes 3 and 6) programmed lysates and 32p-labeled WT (lanes 1-3) or m2 (lanes 4-6) -137/-64 IL-2Rα oligonucleotides. (B) EMSAs were performed with 32p-labeled -137/-64 m2 oligonucleotide and 1.5 μl of in vitro translated Elf-1 protein (lanes 1-6) or ERGβ protein (lanes 7-12). In lanes 2-6 and 8-12, increasing amounts of in vitro translated ERGβ or Elf-1 protein were added, respectively. The total amount of lysate added in each lane was kept constant by adding unprogrammed rabbit reticulocyte lysate.

Figure 4. ERGβ/Fli-1 can compete with Elf-1 for binding to the high affinity m2 Elf-1 site but cannot bind the WT IL-2Rα Elf-1 site. (A) EMSAs were performed with unprogrammed (lanes 1 and 4), ERGβ (lanes 2 and 5) or Elf-1 (lanes 3 and 6) programmed lysates and 32p-labeled WT (lanes 1-3) or m2 (lanes 4-6) -137/-64 IL-2Rα oligonucleotides. (B) EMSAs were performed with 32p-labeled -137/-64 m2 oligonucleotide and 1.5 μl of in vitro translated Elf-1 protein (lanes 1-6) or ERGβ protein (lanes 7-12). In lanes 2-6 and 8-12, increasing amounts of in vitro translated ERGβ or Elf-1 protein were added, respectively. The total amount of lysate added in each lane was kept constant by adding unprogrammed rabbit reticulocyte lysate.

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Address correspondence to Dr. Warren J. Leonard, Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

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