Cooperation between Core Binding Factor and Adjacent Promoter Elements Contributes to the Tissue-specific Expression of Interleukin-3*

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Tissue-specific expression of interleukin-3 (IL-3) is mediated via cis-acting elements located within 315 base pairs of the transcription start site. This is achieved in part through the positive activities of the AP-1 and Elf-1 sites in the IL-3 promoter. The contribution to T cell-specific expression by other promoter sites was assessed in a transient expression assay with IL-3 promoter constructs linked to a luciferase gene, focusing initially on the core binding factor (CBF) site, which is footprinted in vivo upon T cell activation. Activity of the CBF site is shown to be critically dependent on the adjacent activator site Act-1 and CBF sites form a functional unit (AC unit) with dual activity. The AC unit is demonstrated to enhance basal activity of promoters both in fibroblasts and T cells. This activity is further inducible in activated T cells, but not in fibroblasts. In addition to the already identified NIP repressor site, evidence is presented for a second repressor region that restricts promoter activity in fibroblasts. Finally, a novel positive regulatory element has been mapped in the IL-3 promoter between nucleotide -180 and -210 that leads to increased expression in T cells. Together these results demonstrate that T cell expression of IL-3 is not specified by the activity of a single tissue-specific element, but instead involves multiple interacting elements that provide both specific positive regulation in T cells and specific negative regulation in fibroblasts.

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The abbreviations used are: IL-3, interleukin-3; bp, base pairs; CBF, core binding factor; GM-CSF, granulocyte-macrophage-colony-stimulating factor; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; nt, nucleotide; DMEM, Dulbecco's modified Eagle's medium; TPA, 12-O-tetradecanoylphorbol-13-acetate; AC unit, Act-1 and CBF sites form a functional unit; RLU, relative light units; NIP, nuclear inhibitor protein; NFAT, nuclear factor of activated T cells.

Interleukin-3 (IL-3)* is involved in the proliferation and differentiation of hematopoietic progenitor cells (1). Consistent with its potent biologic activity, IL-3 gene expression is highly regulated and is restricted to human T and NK cells (2). Therefore, the IL-3 promoter has been extensively studied, and multiple regulatory elements have been identified within 315 bp of the transcription start site (3-6). These elements include several activator sites: AP-1, Elf-1, and Act-1 (NFIL3); a repressor site, NIP; and a "permissive" site that binds core binding factor (CBF).

The activity of individual regulatory elements has been extensively studied in the context of multiple promoters/enhancers (7-11). However, the emphasis on identifying novel regulatory elements has prevented a complete understanding of the functional interactions between elements already identified. These interactions are important as they represent another possible level of regulatory activity and may in part specify the differential expression of genes despite impressive similarities of their promoters. For example, the promoter for both granulocyte-macrophage-colony-stimulating factor (GM-CSF) and IL-3 bind many similar proteins but exhibit different patterns of expression, with GM-CSF being produced by fibroblasts, macrophages, and endothelial cells (4, 5, 8, 10). Therefore, the activity of a particular site may vary according to the context within which it is found, as adjoining regulatory sites may modulate its activity (4, 12).

The IL-3 promoter is well suited to address possible functional interactions between different regulatory elements, since a relatively small region of DNA contains several well characterized sites that are important outside the context of IL-3 regulation. We initially focused on the normal function of CBF, since mutations of this protein complex are associated with specific subtypes of acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) (13-17). In the present study we demonstrate that the CBF site and adjacent Act-1 element form a regulatory unit that provides inducible promoter activity in Jurkat T cells, but not in NIH3T3 fibroblasts. We also demonstrate that additional upstream portions of the IL-3 promoter inhibit promoter activity in NIH3T3 cells. Finally, an initial characterization of a previously unknown positive regulatory region of the IL-3 promoter between nt -180 and nt -207 is provided.

MATERIALS AND METHODS

Cell Culture—The human Jurkat T cell line E6.1 and NIH3T3 cells were obtained from ATCC and maintained in 5% CO2 at 37°C. Jurkat T cells were cultured in RPMI medium supplemented with penicillin (50 units/ml), streptomycin (50 μg/ml), and 10% fetal calf serum (RPMI complete). NIH3T3 cells were cultured in DMEM supplemented with penicillin, streptomycin, 1 mM sodium pyruvate, and 10% fetal calf serum (DMEM complete). Jurkat cells were stimulated with a combination of TPA (10 ng/ml) and ionomycin (0.5 μM). NIH3T3 cells were stimulated with TPA alone.

IL-3 Promoter Constructs—Isolation of the -315-bp IL-3 promoter and insertion into an IL-3 reporter gene has already been described (18). Similarly, G-C mutation of the CBF site of the IL-3 promoter has been described (18). Briefly, guanine -139, -137, and -136 within the CBF binding site were selectively mutated to cytosine using standard polymerase chain reaction methodology. This mutation abrogates CBF protein binding. Flanking HindIII sites were added to the full-length -315-bp promoter, or G-C mutant (-315GC), to the luciferase gene.
erase reporter gene previously described by DeWet et al. (−315pL, −315/GCpL) (19). An independent, higher yield luciferase reporter gene (pFlash*) was derived from the pFlash vector (Synapsys Corp., Burlington, MA) by excision of the downstream polylinker between Apal and SmaI, followed by replacement of the SstI-XbaI (luciferase nt 57) pFlash upstream polylinker with the equivalent fragment from the luciferase reporter gene, pXp1 (pFlash*). The IL-3 promoter was then inserted at the new upstream HindII site (−315pF*/−315/GCpF*). All reporter genes used in a particular experiment were derived from the same root plasmid. Specific constructs are depicted schematically within individual figures and were derived as follows.

A second CBF site (CBFp*) was created at nt −192 using standard polymerase chain reaction methodology to introduce a C-G and T-G replacement of the IL-3 promoter upstream of nt −180 (3a) or nt −189, respectively. Replacement of the DNA fragment between nt −180 and nt −207 with an AC unit was done via two intermediate cloning steps. The resultant promoter had an inserted oligonucleotide, 5′-ataggATGAATAATTACGTCTGTGGTTTtccc-3′ , that contained an AC unit (upper case letters) in place of the 27-bp MsdI (nt −207)–Sau3a (nt −180) fragment in the −315pL promoter vector (−315NA/CpL). The equivalent replacement was made in an IL-3 promoter containing a G-C mutation at the endogenous CBF site (−315NACpL). The equivalent replacement was made in an IL-3 vector (I−XbaI) by excision of the downstream polylinker between SstI and SmaI (Stratagene, La Jolla, CA) or by electroporation in serum-free conditions (20, 21). Therefore, it is an unreliable method for normalization. It has also been our experience that spectrophotometry has not quantitated plasmid DNA consistently with the methods proposed by others (21). Using this procedure, replicate transfections using independent isolates of the same luciferase construct have yielded differences in luciferase activity of <10%. This is more consistent, and accurate, than normalizing data to the independently variable activity of a co-transfected second construct. Luciferase activity was measured as relative light units (RLU) (Mono- light Luminometer model 2010). All data are presented as the mean relative luciferase activity as calculated by the formula (RLUtest construct/RLUreference construct). Error Bars indicate 1 standard deviation of the mean relative luciferase activity.

Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared from either Jurkat T cells or NIH3T3 cells using the Andrews procedure and were used in gel shift assays as described (22). Labeling of the oligonucleotide probe was accomplished either by filling the 5′ ends using DNA polymerase large fragment (Klenow) in the presence of dATP, dGTP, dTTP, and [α-32P]dCTP (3,000 Ci/mmol; DuPont NEN). Labeled probes were purified by gel electrophoresis. A 50–100 molar excess of competitor oligonucleotides was added to the binding reaction 15–20 min prior to the probe. The AP-1-specific oligonucleotide was kindly provided by N. C. Andrews. Antiserum specific for NFATp and Oct-1 was kindly provided by A. Rao and G. R. Crabtree, respectively.

RESULTS
The CBF Site within the Human IL-3 Promoter Is Position-sensitive—We previously demonstrated that CBF is necessary for IL-3 promoter activity (3). To further characterize the functional role of CBF, a new site (CBFp*) was introduced 51 bp upstream of the endogenous location and tested for its capacity to rescue the activity of an IL-3 promoter mutated at the resident CBF site. These experiments were carried out in the human Jurkat T cell line J E6.1. As indicated in Fig. 1A, there is little promoter activity in unstimulated Jurkat cells 2 D. S. Taylor, unpublished data.
Functional Role of CBF in the Regulation of Interleukin-3

The Act-1 and CBF Sites Form a Functional Unit—The endogenous CBF site within the IL-3 promoter is interesting as it is juxtaposed to another important regulatory element, Act-1. Such juxtaposition of CBF and another regulatory site is a recurring motif in other promoter/enhancer elements (Table II), and suggests a functional interdependence between the CBF and Act-1 sites in the context of the IL-3 promoter.

To evaluate the functional interdependence of the Act-1 and CBF sites, a second Act-1/CFB (AC) unit, or a mutant thereof, was introduced upstream of the wild type site at nt –180. Introduction of either the wild type, or one of several mutants of the AC unit, did not involve the deletion of endogenous nucleotides and resulted in extension of the IL-3 promoter by 34–42 bp. In contrast to our previous experiments with promoters containing the *CBF site (–315*CBFpL), the AC unit significantly up-regulates promoter activity (Fig. 2) in Jurkat T cells upon stimulation with TPA and ionomycin. Insertion of this unit also resulted in a tendency to increase the basal (constitutive/unstimulated) activity of the IL-3 promoter. In addition, the inserted AC unit overcomes the inhibition of promoter activity caused by a G-C mutation of the endogenous CBF site. Mutation of either the Act-1 or CBF component of the AC unit resulted in a sharp decrease in promoter activity. Thus, in Jurkat T cells the Act-1 and CBF sites function only as a unit (not individually) to provide inducible promoter activity.

The AC Unit Is Functional in a Minimal Promoter—Having established the functional interdependence of the Act-1 and CBF sites, we next evaluated its function outside the context of an intact, full-length proximal IL-3 promoter. To this end, a normal AC unit or an AC unit containing a mutant CBF site was inserted 31 bp upstream of the wild type site at nt –180. Introduction of either the wild type, or one of several mutants of the AC unit, did not involve the deletion of endogenous nucleotides and resulted in extension of the IL-3 promoter by 34–42 bp. In contrast to our previous experiments with promoters containing the *CBF site (–315*CBFpL), the AC unit significantly up-regulates promoter activity (Fig. 2) in Jurkat T cells upon stimulation with TPA and ionomycin. Insertion of this unit also resulted in a tendency to increase the basal (constitutive/unstimulated) activity of the IL-3 promoter. In addition, the inserted AC unit overcomes the inhibition of promoter activity caused by a G-C mutation of the endogenous CBF site. Mutation of either the Act-1 or CBF component of the AC unit resulted in a sharp decrease in promoter activity. Thus, in Jurkat T cells the Act-1 and CBF sites function only as a unit (not individually) to provide inducible promoter activity.

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An AC Unit Is Functional in Heterologous Cells—The activity of the full-length, –315 bp proximal IL-3 promoter is highly tissue restricted. It is functional only in human T and NK cells (2). Such observed tissue-specific activity may result from specific positive regulation in a permissive cell type, specific inhibition in nonpermissive cells, or a combination thereof. Previous work in this laboratory identified a T cell-specific complex that, upon activation, binds the 5' portion of the Act-1 region, suggesting that the AC unit may contribute to the observed activity of the full-length, –315 bp proximal IL-3 promoter.

Fig. 1. *CBF only partially overcomes CBF (G-C) mutation. A, Jurkat T-cells were transfected with the individual promoter constructs depicted schematically in the lower portion of the panel. Single base mutations were introduced to create the *CBF site and are indicated in boldface type. Sixteen hours after transfection cells were mock-stimulated or stimulated with a combination of TPA and ionomycin. Six hours after stimulation luciferase activity was measured in cell lysates. Luciferase activity was measured as RLU. The data from three independent experiments are presented as the mean relative luciferase activity ± 1 standard deviation of the mean relative luciferase activity. The stimulated wild type IL-3 promoter (–315pL) is the reference construct in this experiment and has a relative luciferase activity of 1.0. B, nuclear extracts were prepared from Jurkat T cells. The nuclear extracts were incubated with the indicated competitor oligonucleotide and an oligonucleotide probe corresponding to either the endogenous CBF site or the newly introduced CBF site (*CBF). The CBF binding site is indicated by a bracket.
However, the AC unit increases the stimulation index (sorelementmapstothetupstreamregionoftheIL-3promoter, (unstimulated)promoterasnotedinFig.2).AstheNIPrepres-
in elongation of the promoter and a tendency to increase basal
unit into the full-length proximal IL-3 promoter resulted both
180 and nt
207 of the IL-3 Promoter—
result from more than one closely migrating complex and sug-
more diffuse appearance of this complex in NIH3T3 cells may
present in nuclear extracts from Jurkat T cells (Fig. 4). The CBF segment of the AC unit bindsa
NIH3T3cells (Fig. 4). The CBF segment of the AC unit binds a
major complex in NIH3T3 cells that migrates and competes
identically to the previously identified CBF protein complex
in NIH3T3 cells (Fig. 4). The CBF segment of the AC unit binds a
migrating complex present in both cell types (Fig. 4B). This
band was previously identified in this laboratory as Oct-1 or an
Oct-1-like protein. It is possible that the faster migrating com-
plex that is specifically competed by Act-1 in the Jurkat nuclear
extract represents the recently identified E4BP4 protein,
NFIL3A (25).

A Positive Regulatory Element (CK3) Is Present between nt
180 and nt -207 of the IL-3 Promoter—Insertion of the AC
unit into the full-length proximal IL-3 promoter resulted both
in elongation of the promoter and a tendency to increase basal
(unstimulated) promoter as noted in Fig. 2. As the NIP repres-
sor element maps to the upstream region of the IL-3 promoter,
it was possible that the inserted nucleotides diminished its
effect by increasing the distance between NIP and other im-
portant downstream elements. To investigate this possibility,
the promoter fragment between nt -180 and nt -207 was
deleted and replaced with an AC unit. This region was chosen
as it contained no previously identified promoter elements. As
shown in Fig. 5, constancy of the distance between NIP and
other downstream elements of the IL-3 promoter restores a low
basal activity of the IL-3 promoter in the presence of an addi-
tional AC unit. Furthermore, mutation of the endogenous CBF
is fully rescued in the context of the upstream AC unit (data not
shown). However, deletion of the region between nt -180 and
nt -207 causes a decrease in overall promoter activity, sug-
gesting that a previously unidentified positive regulatory ele-
ment is perturbed in this promoter construct.

To further analyze this region, the activity of a -180-bp IL-3
promoter was compared with the -207 and -315 IL-3 pro-
moter constructs in both Jurkat and NIH3T3 cells (Fig. 6). In
all experiments performed, regardless of cell type, inclusion of
the 27 bp between nt -180 and nt -207 induced a significant
increase in promoter activity.

Two Specific Complexes Are Bound by the CK3 Element—The
coding strand of DNA between nt -180 and nt -207 bears no
homology to known regulatory elements. However, the noncod-
inc strand contains sequences that are similar, but not identi-
cal, to known binding sites for nuclear factor of activated T cells
(NFAT), AP-1, and the 3' portion of Act-1 (Table III). To inves-
tigate protein binding to the IL-3 promoter in this region (CK3),
gel shift assays were performed using as a probe both a restric-
tion fragment of the IL-3 promoter containing nucleotides
(MseI-Sau3a) and a synthetic oligonucleotide containing the
nucleotides of interest (Fig. 7A). Both probes bind a faster
migrating complex (CK3a) and a slower migrating doublet
(CK3b).

The faster migrating protein complex (CK3a) is specifically
competed by unlabeled "self" oligonucleotide and nucleotides
containing the complete Act-1 site, but is not competed by an
oligonucleotide containing only the 5' portion of the Act-1 site

TABLE II

| Promoter | Sequence |
|---------|----------|
| IL-3    | ATGAATAATACGCTGCGGTTTT | Act-1-CBF |
| TCR-β   | CAGAGCGGGTTT | Ets-CBF |
| MoMLV   | CCCATTAATCATTTCCTCT | CBF-Cleo(AP/Ets) |
| GM-CSF  | TCGGTCACATTTGATCTCCTCT | |

Fig. 2. Act-1 and CBF form a func-
tional unit and overcomes a CBF
(G-C) mutation. Jurkat T cells were
transfected with the individual promoter
constructs depicted schematically on the
left portion of the figure. 16 h after trans-
faction cells were mock-stimulated or
stimulated with a combination of TPA
and ionomycin. 6 h after stimulation lu-
ciferase activity was measured in cell ly-
sates. Data from three independent ex-
periments are presented as outlined in
Fig. 1. The stimulated wild type IL-3 pro-
moter (top construct) is the reference con-
struct in this experiment and has a rela-
tive luciferase activity of 1.0.
The recent identification of mutant CBF proteins created by the t(8;21), t(3;21), and inv(16) chromosomal rearrangements and the association of these rearrangements with specific subtypes of AML or MDS has encouraged intense study of the functional role of CBF in the regulation of interleukin-3.

**DISCUSSION**

IL-3 is a lymphokine important for normal hematopoiesis (1). Its expression is highly tissue-specific, being expressed primarily in T and NK cells (2). Therefore, its promoter has been extensively studied (3–6). Most recently, in vivo footprinting of the IL-3 promoter identified a CBF binding site as one of the few regions specifically protected upon stimulation of T cells (3). The initial characterization of this site indicated that CBF binding to the IL-3 promoter is necessary, but not sufficient, for IL-3 promoter activity (3).

The present data demonstrate that the CBF complex cannot function alone. This is supported by the observation that a second CBF binding site (CBF) that binds the CBF complex in a gel shift assay provides neither positive promoter activity nor overcomes a mutation of the endogenous CBF site (Fig. 1). In contrast, a CBF site in conjunction with the adjacent Act-1 region of the IL-3 promoter both augments promoter activity and overcomes a null mutation of the endogenous CBF site. Both effects are abrogated by mutation of either the Act-1 or CBF component of the unit (Fig. 2). The AC unit functions similarly in the context of a minimal (−61 bp) IL-3 promoter (Fig. 3). Thus, the AC unit is an autologous functional unit that retains its activity outside the context of a full-length IL-3 promoter and requires both the Act-1 and CBF sites to be intact.

The AC unit functions in both human T cells (Jurkat) and murine fibroblasts (NIH3T3), and nuclear extracts from both cell types contain proteins that bind similarly to both the Act-1 and CBF sites of the AC unit (Fig. 4). In the context of T cells, the AC unit augments both basal (constitutive/unstimulated) and inducible (stimulated) promoter activity. However, the augmentation of inducible promoter activity in T cells is greater than the augmentation of basal activity as indicated by an increase in the stimulation index of an IL-3 promoter containing a second AC unit (Figs. 2 and 3). This effect is not
observed in NIH3T3 cells (Fig. 3). Thus, the AC unit contributes to the tissue-specific expression of IL-3 by its selective positive contribution to inducible promoter activity in the context of T cells.

The mechanism underlying the interdependence of the Act-1 and CBF sites is unknown. As shown for other DNA binding factors, it may involve alteration in DNA configuration via bending (27). Alternatively, CBF may function as a chaperon protein and augment/inhibit binding of other proteins to the promoter. For example, several isoforms of CBF have been identified and found to have variable activity in vitro (24, 28). Thus, the capacity of CBF to modulate the association between other proteins, such as the Act-1-binding proteins, and DNA may be dependent on the relative amount of the various CBF isoforms under various conditions. Previous in vivo footprinting of the IL-3 promoter is consistent with this hypothesis. These data demonstrate protection of the CBF site only upon stimulation, despite similar amounts of CBF binding activity in nuclear extracts obtained from either stimulated or unstimulated Jurkat cells (3). This hypothesis is further supported by recent data that demonstrate a similar functional interdependence between PEBP2a, the murine homologue of CBF, and Ets-1, which binds an adjacent regulatory element in the T cell receptor α chain enhancer (29).

Although the AC unit contributes to the specific up-regulation of the IL-3 promoter activity in T cells, as evidenced by its effect on a minimal promoter, the full-length (−315 bp) IL-3 promoter is not active in fibroblasts. Therefore, additional elements between nt −61 and nt −315 of the IL-3 promoter contribute to tissue-specific promoter activity by limiting activity in non-T cells. As suggested by our results in Fig. 3B, there are at least two elements that inhibit IL-3 promoter activity in NIH3T3 cells. One element resides between nt −173 and nt −315 (Fig. 3B). The presence of this upstream region of the IL-3 promoter is also associated with a low basal activity of the promoter in Jurkat T cells (Fig. 3A). The NIP site of the IL-3 promoter is located at nt −266, is a negative regulator of transcription, and has been extensively studied in this laboratory (5, 30). The relative proximity of the upstream NIP region of the IL-3 promoter to the AP-1 (nucleotide −303) and Elf-1

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**Fig. 4.** The Act-1 and CBF sites of the IL-3 promoter bind similar protein complexes in nuclear extracts from both Jurkat T cells and NIH3T3 fibroblasts. Nuclear extracts were prepared from either unstimulated Jurkat T cells or unstimulated NIH3T3 cells. The nuclear extracts were incubated with the indicated competitor oligonucleotide and an oligonucleotide probe corresponding to either the endogenous CBF (A) or endogenous Act-1 (B) site. A, the arrow indicates the CBF protein complex, and the bracket indicates the CBF binding site. B, the arrow indicates the Oct-1 protein complex. The boxed nucleotides of the Act-1 probe correspond to the previously defined Act-1 region of the IL-3 promoter. Brackets indicate the Oct-1 and E4BP4/NFIL3A binding sites.

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**Fig. 5.** Replacement of IL-3 promoter between nt −180 and nt −207 decreases promoter activity. Jurkat T cells were transfected with the indicated promoter constructs and stimulated as described in Fig. 1. The NAC constructs contain a second AC unit and flanking nucleotides that replaces the endogenous IL-3 promoter between nt −207 and nt −180. Data from two independent experiments are presented as outlined in Fig. 1. The stimulated wild type −315-bp IL-3 promoter (second construct) is the reference construct in this experiment and has a relative luciferase activity of 1.0.
Fig. 6. Positive regulatory activity maps between nt −180 and nt −207. Jurkat T cells (A) and NIH3T3 cells (B) were transfected with the indicated promoter constructs and stimulated as described in Fig. 3. Data from four independent experiments are presented as outlined in Fig. 1. The stimulated −207-bp IL-3 promoter is the reference construct in this experiment and has a relative luciferase activity of 1.0.

The second observation resulting from the study of the AC unit, and immediately relevant to IL-3 promoter function, is the identification of a previously unknown positive regulatory region between nt −2180 and nt −2207 (Fig. 6). This region of the IL-3 promoter is 67–100% conserved between human, new world monkey, sheep, rat, and mice (32). Furthermore, the majority of differences within these 27 bp represent conservative changes. Preliminary characterization of the −2180/−2207 region by gel shift analysis demonstrates that at least two protein complexes bind independently to this region. Although there are sequence similarities between the noncoding strand of this region and the Act-1/Oct-1, AP-1, and NFATp sites, preliminary experiments suggest that these factors are not involved in binding to these 27 nucleotides. In addition to Oct-1, the E4BP4 protein, NFIL3, binds to the 3′ portion of the Act-1 site (6, 33). Although the 3′ portion of the Act-1 site is important for competition of CK3a binding, the sequence of the CK3a site contains only 5 (5′-ATTAC-) of 10 nucleotides that define the E4BP4 consensus site (25). Antiserum specific for the NFIL3 protein is not available, so the relationship between E4BP4/NFIL3 and the observed binding at the CK3a region is unknown at this time. Regardless, a more complete mutational analysis of this region will be necessary to confirm that the protein complexes identified by this gel shift analysis are as-

Table III

| Promoter element | Sequence |
|------------------|----------|
| IL3 AP1 (-303)   | TGAGTCA  |
| IL3 Act-1 (-155) | ATGAATAATTACGTCTG |
| IL3 CK3a         | TTAGTTA |
| NFIL2E (-282NFAT)| GGAGGGAAAACGTTTCAT |
| GM550 (NFAT-AP1) | GAAAGGGAAGAGCAAGTCAT |
| IL3 CK3b         | GAAACAGAAAGAGAGA |

(nucleotide −288) elements is intriguing. The latter two regions are known positive regulators of promoter activity, and Elf-1 has been implicated in the specific positive regulation of the IL-3 promoter in T cells (4). Thus, the AP-1-Ef1-11IP complex may represent a second example within the IL-3 promoter of a functional interdependence between adjacent regulatory regions. A second element is present between nt −61 and nt −173. This element functionally negates the positive contribution of the endogenous AC unit, which is evidenced by a decrease in activity of the −173-bp promoter as compared with the −61-bp promoter containing an AC unit. A highly conserved CK1/CK2 DNA sequence is present in this region and may be important in this activity, since a concatamer of this sequence has been shown to inhibit the basal transcription of a heterologous promoter and proteins from the transcriptionally active Rel/NFκB family bind to the CK1 element (31). The combined effect of these two negative elements is abrogation of IL-3 promoter activity in the context of NIH3T3 cells.

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FIG. 7. The IL-3 promoter between nt −180 and nt −207 contains two protein binding regions. Nuclear extracts were prepared from J urkat T cells that were stimulated for 6 h with a combination of TPA and ionomycin. A, the extracts were incubated with the competitor oligonucleotide indicated below each lane and one of three probes indicated below the lane (AP1, 5′-tgaaacacctgtgcTGAGTCActggag; A TPAl and ionomycin). Tctagagct; Act, catggATGAATAATTACGTCTGca; Act-5

 IL3NFAT, agctTGAAACAAGAAAAAAG; IL2NFAT, agcttGAAAGGACAGAAAAAAGATTACTTAg.

Sau 3a, TTAAGTAATCTTTTTTCTTGTTTCACT; or CK3, agctTGAAA-

IL-3 promoter. Relevant binding sites within oligonucleotides derived from exogenous promoters are also noted in uppercase letters. Associated with the positive regulatory activity. These studies are in progress.

In conclusion, we demonstrate that the Act-1 and CBF sites of the IL-3 promoter form a functional unit. We further demonstrate that the tissue-specific activity of the proximal IL-3 promoter results from the combined activity of this unit and other regulatory elements within the promoter rather than from a single, tissue-specific protein. The contribution of the Act-1/CFB unit to this activity is important as it mediates inducible promoter activity in the context of T cells (but not fibroblasts). It remains important to determine how, or whether, mutant forms of the CBF protein, relevant to AML and MDS, alter this tissue-specific activity. However, the recognized association of the M4Eo subtype of AML with both a mutation in the β-chain of CBF and eosinophilia indirectly suggests that IL-3, a positive regulator of eosinophil differentiation, is abnormally up-regulated in these cells.

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