Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9650–9654, December 1990
Biochemistry

Transcriptional regulation of interleukin 3 gene expression in T lymphocytes

(transcription factor AP-1/DNA binding proteins/transcription factor NF-IL3-A)

SARA G. SHOEMAKER, ROBERT HROMAS, AND KENNETH KAUSHANSKY*

Department of Medicine, Division of Hematology at the University of Washington, Seattle, WA 98195

Communicated by Earl W. Davie, September 6, 1990 (received for review April 18, 1990)

ABSTRACT **Interleukin 3 (IL-3 or multi-colony-stimulating factor) plays an important role in the hematopoietic response to inflammatory stimuli through its action on both immature and mature blood cells. Like other lymphokines, IL-3 is produced in response to activation of the T-cell receptor and protein kinase C pathways. By using nuclear run-on assays of quiescent and stimulated T-cell lines, we demonstrate that IL-3 gene expression is controlled, at least in part, at the level of transcription. Functional reporter gene analysis was used to delineate two regions of the IL-3 5′ flanking sequence responsible for transcriptional stimulation. DNA binding proteins that potentially mediate these responses were then recognized by mobility-shift and DNase footprinting assays. One region responsible for transcriptional enhancement was localized to the sequence GATGAATAAT, the cognate site of a transcription factor, here termed NF-IL3-A. A second region of functional activity and protein binding was localized to a single transcription factor AP-1 site. In addition three functionally inhibitory regions were identified. These results, along with the further characterization of NF-IL3-A, will contribute to the understanding of IL-3 gene regulation in stimulated T cells.**

Among the growth factors involved in hematopoiesis, interleukin 3 (IL-3 or multi-colony-stimulating factor) affects the widest variety of cell types. In conjunction with other growth factors, IL-3 acts on marrow-derived progenitor cells to stimulate the development of erythrocytes, granulocytes, macrophages, and megakaryocytes (1, 2). In addition, it influences the growth and activation of lymphocytes (3), promotes the self-renewal of hematopoietic progenitor cells (4), and acts upon mature leukocytes to enhance their functional activity (5, 6). In this way, IL-3 is thought to be involved in the host response to infectious, inflammatory, and immunologic stimuli.

Constitutive expression of IL-3 has not been observed in any normal cell yet examined. By using the polymerase chain reaction, mRNA specific for human IL-3 has been identified in a number of cell sources (7), but IL-3 mRNA has only been found in T lymphocytes or T-cell lines treated with stimulators of the T-cell receptor or of the protein kinase C pathway (8). The molecular basis for this highly inducible and selective tissue specificity is uncertain.

To further understand the regulation of IL-3 production, we have cloned the gene for human IL-3 and have performed a detailed analysis of the 682-base-pair (bp) region upstream of the start of IL-3 transcription. Our results suggest that IL-3 gene expression is controlled, at least in part, at the level of transcription and that binding of trans-acting factors at two distinct sites mediate much of this response.

MATERIALS AND METHODS

Cell Lines and Culture. The T-lymphocyte cell line Jurkat (ATCC), the cervical carcinoma cell line HeLa (ATCC), and the leukemia cell line AML-193 were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum, glutamate, and antibiotics. The gibbon T-lymphocyte cell line MLA-144 (ATCC) was maintained in RPMI 1640 medium supplemented as noted above. AML-193 cells were also supplemented with recombinant human granulocyte/macrophage colony-stimulating factor.

Northern Blot Analysis. Whole cellular RNA was enriched for poly(A)-containing transcripts by chromatography on oligo(dT)-cellulose, size-fractionated by formaldehyde/agarose gel electrophoresis, and transferred to nitrocellulose (9). The blots were hybridized with full-length cDNA probes for gibbon IL-3 (10), murine c-*jun* (11), human cAMP-responsive element binding protein (CREB) (12), bovine β-actin (13), and a 1.1-kilobase fragment containing the first exon of the human c-*fos* gene (14), all labeled by random priming (Amersham), or with oligonucleotides for human *JUN-B* [bases 709–738 (sequence kindly provided by Richard Turner, University of California, San Francisco)] and human CRE-BP-1 [bases 73–96 (15)] labeled with [γ-^{32}P]ATP by polynucleotide kinase. The blots were washed twice at 20°C and twice at 65°C in 0.2× SSC/0.1% $NaDodSO_4$ (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) for the cDNA and genomic probes or in 3 M tetramethylammonium chloride at 68°C or 62°C for the 30- or 24-bp oligonucleotide probes, respectively (16).

Nuclear Run-On Analysis. Cell lines were grown to 1×10^6 cells per ml and then stimulated with concanavalin A (Con A; Boehringer Mannheim) at 10 μg/ml and phorbol 12-myrisitate 13-acetate (PMA; Sigma) (100 ng/ml) for 15 min to 24 hr. Cells were washed and lysed, and labeled run-on transcripts were produced from the resultant nuclei as described (9). Antisense single-stranded cDNA for gibbon IL-3 (10), human granulocyte/macrophage-colony-stimulating factor (17), human β-actin (18), and M13 were slot-blotted, prehybridized, and then hybridized for 72 hr at 65°C with an equal amount of radioactivity of each of the run-on RNAs (9). The blots were washed twice at 20°C and twice at 65°C in 0.1× SSC/0.1% $NaDodSO_4$ and then exposed to film.

Construction of Hybrid Genes. A 40-base antisense oligonucleotide derived from a published IL-3 cDNA sequence (bases 73–112; ref. 10) was used to screen a human genomic fetal liver library in λ Charon 4A, as described (19) with the exception that 3 M tetramethylammonium chloride at 73°C was used to set the wash stringency (16). A full-length clone of the human IL-3 gene was thus obtained. A 925-bp *Pst* I fragment containing the 5′ flanking region of the gene was then subcloned into M13 (termed IL-3pr), sequenced by the dideoxynucleotide chain-termination method and a *Bam*HI site was introduced 42 bp downstream of the site of transcription initiation by site-directed mutagenesis (17). By

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL-3, interleukin 3; Con A, concanavalin A; PMA, phorbol 12-myrisitate 13-acetate; CAT, chloramphenicol acetyltransferase; CREB, cAMP-responsive element binding protein.

*To whom reprint requests should be addressed.

using this site, fragments of the 5′ IL-3 flanking region of successively larger size were linked to a clone of the bacterial chloramphenicol acetyltransferase (CAT) gene. These promoter–reporter constructs were assembled in pUC18 by using standard techniques and were numbered according to the nucleotide size of the promoter construct counting from the cap site. All constructions were sequenced for confirmation.

Electroporation and Functional Analysis. Cells were washed and resuspended in Hepes-buffered saline (30 mM Hepes, pH 7.05/137 mM NaCl/5 mM KCl/10 mM sodium phosphate) at 1×10^7 cells per ml along with 20 μg of each construct DNA and 10 μg of the plasmid vector RSV-β-Gal (to provide a control for the relative efficiency of transfection). Electroporation was carried out using the Cellporator device (BRL) with 750 V/cm for 9 msec at 20°C. The cells were then divided and cultured in control medium or medium supplemented with Con A (10 μg/ml) and PMA (100 ng/ml). After culture for 1 (MLA) or 3 (Jurkat) days, cell lysates were assayed for CAT and β-galactosidase activity and calculated as described (9).

Mobility-Shift and DNase I Footprinting Assays. Nuclear extracts were prepared according to Dignam *et al.* (20). Duplex oligonucleotides representing various regions of the IL-3 promoter (see Fig. 2*B*) were end-labeled with [γ-^{32}P]-ATP by using polynucleotide kinase. Nuclear extract (10 μg) or purified recombinant c-jun protein (20 ng) was preincubated with 50 ng of competitor duplex oligonucleotide in 10 μl of binding buffer [10 mM Hepes, pH 7.9/50 mM NaCl/3% (vol/vol) glycerol/1.25 mM EDTA/2 mM dithiothreitol/poly(dI-dC) (0.1 mg/ml)] for 15 min and then 1 ng of ^{32}P-labeled duplex oligonucleotide was added and the reaction was incubated for an additional 20 min at 20°C. The reaction products were fractionated by electrophoresis through 4% polyacrylamide gels (50 mM Tris, pH 8.3/400 mM glycine/2 mM EDTA) at 100 V. The gels were dried and exposed to film. DNase I (Worthington) protection assays were performed as described (21) in 50-μl reaction mixtures containing mobility-shift assay buffer, 25 μg of nuclear extract, and 1 ng of a *Stu* I–*Sma* I (positions −313 to −59) fragment of the IL-3 promoter end-labeled with [γ-^{32}P]ATP. Digestion products were size-fractionated on 6% acrylamide/urea sequencing gels adjacent to sequencing reaction products.

RESULTS

Nuclear Run-On Analysis. To determine the mechanisms that contribute to the regulation of IL-3 gene expression, Northern blot analysis was performed on Jurkat and MLA cells. IL-3-specific transcripts were detected within 90 min to 6 hr of mitogenic stimulation (Fig. 1). By using nuclear run-on analysis, both T-cell lines were observed to actively transcribe the IL-3 gene within 2 hr of stimulation. Transcription appeared to peak at 6 hr but was still present at 24 hr.

Functional Analysis of the 5′ Region of the IL-3 Gene. To identify regions within the IL-3 gene of possible functional significance, homology searches were performed using 682 bp of the IL-3 5′ flanking region. Several sequences completely or partially matching the binding sites of characterized transcription proteins were detected (Fig. 2*A*). To determine the functional significance of these sequences for expression of the IL-3 gene, reporter gene analysis was next performed.

As shown in Fig. 2*B*, constructs containing 161 or 313 bp of the IL-3 5′ flanking sequence lead to the highest reporter gene activities in stimulated Jurkat cells. As little as the 44 bp upstream of the cap site could drive the low level of constitutive promoter activity noted in all of the constructs, but this "promoter" was poorly inducible, resulting in only 7% of the maximal level of reporter gene activity seen in construct 313.

The substantial variations in promoter function among constructs 313 through 126 suggest the presence of several

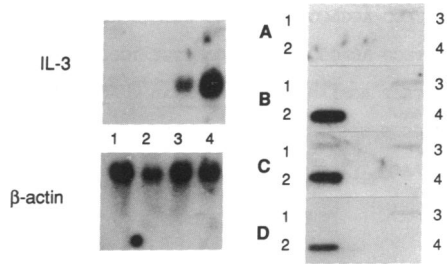

FIG. 1. Northern blot and nuclear run-on analyses of IL-3 gene transcription. (*Left*) Poly(A) RNA (5 μg) from MLA cells (lane 1) or MLA cells stimulated for 15 min, 90 min, or 6 hr with Con A and PMA (lanes 2–4, respectively) was probed with a ^{32}P-labeled cDNA for gibbon IL-3 or bovine β-actin, as indicated. Similar results were obtained with Jurkat cells. (*Right*) ^{32}P-labeled run-on nuclear transcripts were produced from MLA cells (gel A) or from MLA cells stimulated with Con A and PMA for 2 hr, 6 hr, or 24 hr (gels B–D, respectively) and hybridized to antisense single-stranded cDNA for gibbon IL-3 (bands 1), human granulocyte/macrophage-colony-stimulating factor (bands 2), human β-actin (bands 3), or parental phage M13 (bands 4).

regions of functional importance. The stimulated promoter activity noted in construct 126 was increased 5.2-fold when the sequence between positions −126 and −161 was added, a region containing similarities with the binding sites for the transcriptional regulators CREB, NF-IL2-A, and NFAT-1. An additional 2.4-fold increase in reporter gene activity was noted when the region between positions −272 and −313 was added to construct 272. This region contains a transcription factor AP-1 sequence at position −301. A comparable pattern of promoter function was noted in MLA-144 cells but the magnitude of these responses was more prominent (Fig. 2*B*).

The precise location of the first (positions −161 to −126) element and its importance to the functional activity of the promoter was confirmed by mutating the IL-3 flanking sequence between positions −156 and −147 to a random sequence (Fig. 2*B*, construct 356_{NFIL3A^-}). This mutation nearly eliminated inducible reporter gene activity. In contrast, mutation of the CREB binding site immediately downstream of this position increased reporter gene activity (construct 161_{CREB^-}).

To determine whether the two regions that act in cis to augment reporter gene activity confer inducibility upon a heterologous promoter, the 5′ flanking region of IL-3 gene was cloned upstream of the major late promoter of adenovirus (mlp) and the CAT gene. In six experiments, addition of the sequence between positions −313 and −59 upstream of the cap site resulted in a 12.9-fold inducibility of the mlp in response to T-cell stimulation, compared with a 1.6-fold induction with these agents in a construct containing the mlp and CAT alone. No CAT activity was detectable when only the IL-3 sequence and CAT were present.

In addition to the two enhancing regions, three potential negative regulatory sites are also apparent in the IL-3 5′ flanking region. Addition of the regions between positions −313 and −397, positions −161 and −272, and positions −59 and −116 resulted in reproducible decreases in reporter gene activity in stimulated Jurkat cells. Extending the promoter analysis through position −682 resulted in promoter activity similar to that for construct 397 (data not shown).

Nuclear Proteins that Bind to the IL-3 Promoter. Oligonucleotides based upon the sequence of several regions of the human IL-3 gene (Figs. 2 and 3) were tested in mobility-shift assays. Specific retarded bands were consistently produced by extracts from both Jurkat and MLA cells using an oligonucleotide containing the sequence between bases −160 and −130 (Fig. 3*A*). This region shares sequence similarities with the CREB (12, 15) and the IL-2 enhancer elements NFAT-1

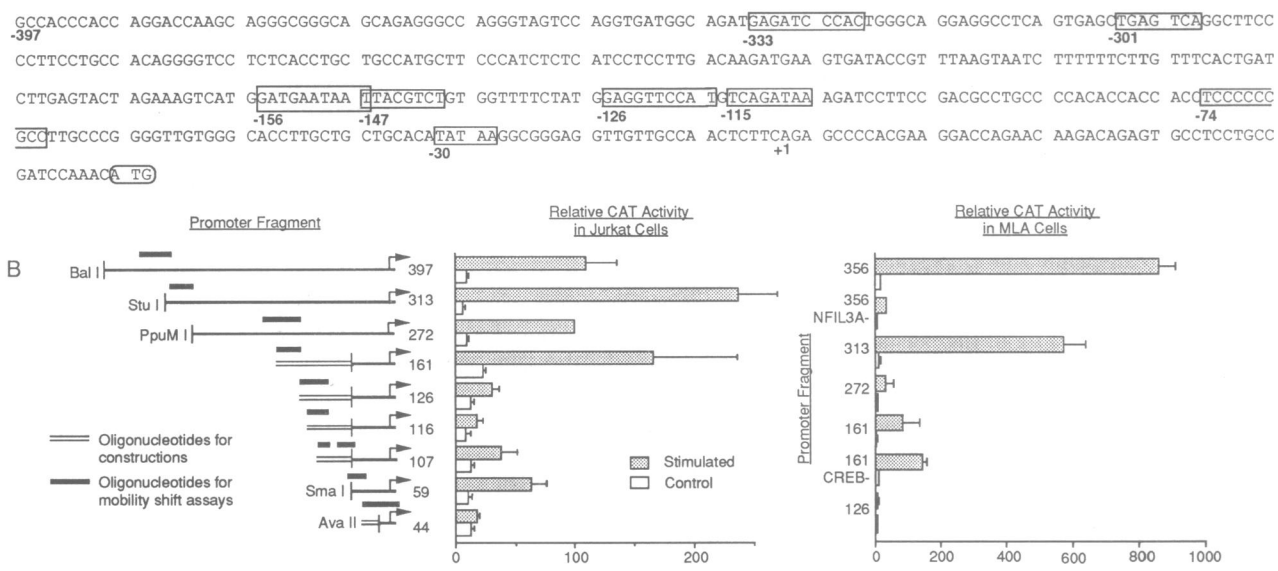

FIG. 2. (*A*) Sequence of the IL-3 5′ flanking region determined from clone IL-3pr. The location of sequences that match or resemble putative and known regulatory regions are noted by boxes and include AP-1 (ref. 11; position −301), CREB (ref. 12; position −147), NFAT-1 (ref. 22; position −156), CK-1 (ref. 23; positions −126 and −333), CK-2 (ref 23; position −115), and AP-2 (ref. 24; positions −74 and −90). The TATA sequence (position −30) and the initiation codon (position +52) are also noted. The site of transcriptional initiation (position +1) was reported by Yang *et al.* (25). (*B*) Functional activity of the IL-3 promoter–CAT constructs in Jurkat and MLA cells. On the left promoter fragments linked to the CAT reporter are mapped. The numbers to the right of the map designate the location of the 5′ end of each construct relative to the cap site. Constructs 397, 313, 272, and 59 utilized available restriction sites (shown at the left), and constructs 161, 161_{CREB^-} (TTACGTCT mutated to TTACGGAT), 126, 116, 107, and 44 utilized synthetic oligonucleotides linked to available restriction sites. Construct 356_{NFIL3A^-} (ATGAATAATT mutated to CACCCGATCG) was generated by site-directed mutagenesis. The relative reporter gene activity (±SEM) is shown for Jurkat and MLA cells, as indicated. The data presented were normalized so as to bring the stimulated level for construct 272 (common to all assays) to a value of 100 and represent the mean of 4–15 experiments for each construction. At least three plasmid preparations have been tested for each construct giving essentially identical reproducible results.

and NF-IL2-A (22, 27). To determine whether the protein(s) that bound to this oligonucleotide represents either of these two families of transcription factors, or instead another DNA binding protein(s), mobility-shift competition experiments were carried out. As shown in Fig. 3*A*, a 50-fold excess of oligonucleotide that binds either of two CREB sites, either of two AP-1 sites, or the NFAT-1 site did not displace the Jurkat protein(s) from the labeled oligonucleotide. An oligonucleotide containing an NF-IL2-A binding sequence competed with an affinity ≈10-fold lower than the specific sequence. In contrast, 1–25 ng of an oligonucleotide that contains mutations at four of the eight CREB-specific bases of the IL-3 5′ flanking region displaces specific oligonucleotide binding with an affinity similar to that of native sequence (Fig. 3, affinity data not shown). Furthermore, Northern blot analysis of poly(A)-containing RNA from Jurkat and MLA cells failed to detect CREB-specific transcripts (data not shown). Additional oligonucleotides containing mutations near the 5′ and 3′ ends of this region were tested for competition and allowed mapping of the binding site between positions −156 and −147 (Fig. 3*B*). To confirm the precise binding site DNase I footprinting was performed. As shown in Fig. 3*C*, nucleotides between 157 and 144 bp upstream of the cap site were protected from digestion in the presence of T-cell nuclear extracts.

A second specific band pattern was obtained using an oligonucleotide pair matching the IL-3 sequence from positions −308 to −276, a region that contains an AP-1 binding site (11). As shown in Fig. 4, stimulated Jurkat and MLA cell nuclei contain AP-1 binding protein(s) that migrate with an electrophoretic mobility identical to that of recombinant c-jun protein. An identical mobility-shift pattern was obtained using an oligonucleotide derived from the AP-1 region of the metallothionein promoter. This banding pattern was specifically displaced by the presence of a 50-fold excess of an unlabeled oligonucleotide that matches the 308/276 sequence only at the AP-1 site but not by irrelevant oligonucleotides or those derived from any other region of the IL-3 promoter. Lower or undetectable levels of this binding activity were present in several preparations of nuclear extracts from unstimulated T-cell lines. Northern blot analysis confirmed the presence of transcripts for the AP-1 proteins c-jun and c-fos in stimulated T cells (Fig. 5).

DISCUSSION

T lymphocytes produce a large number of cytokines that affect the proliferation and activation of cells involved in the inflammatory reaction. The regulation of cytokine gene expression is a complex process that may involve transcriptional, post-transcriptional (typically at the level of RNA stability), or translational mechanisms. In a growing number of instances, transcriptional enhancement has been shown to be responsible for lymphocyte activation (27). By using nuclear run-on analysis, we demonstrate that IL-3 gene expression is regulated, at least in part, by transcriptional enhancement. A functional analysis was utilized to map two regions that substantially up-regulate reporter gene expression in response to mitogenic stimulation of T-lymphocyte cell lines. Thus these elements form an enhancer for a heterologous promoter. By using mobility-shift and DNase footprinting assays, these same regions were found to bind proteins from stimulated T-cell nuclei and to a lesser extent from unstimulated cells.

Although the first of these sites, located between 161 and 126 bp upstream of the IL-3 cap site, contains sequences similar to the binding sites for the transcription factors CREB, NFAT-1, and NF-IL2-A, this region binds an apparently uncharacterized protein based on several criteria. (*i*) Mutation of the CREB site at four of the eight positions within the oligonucleotide probe failed to eliminate specific binding of a nuclear protein to the remainder of the region in mobility-shift assays and inclusion of the same mutation

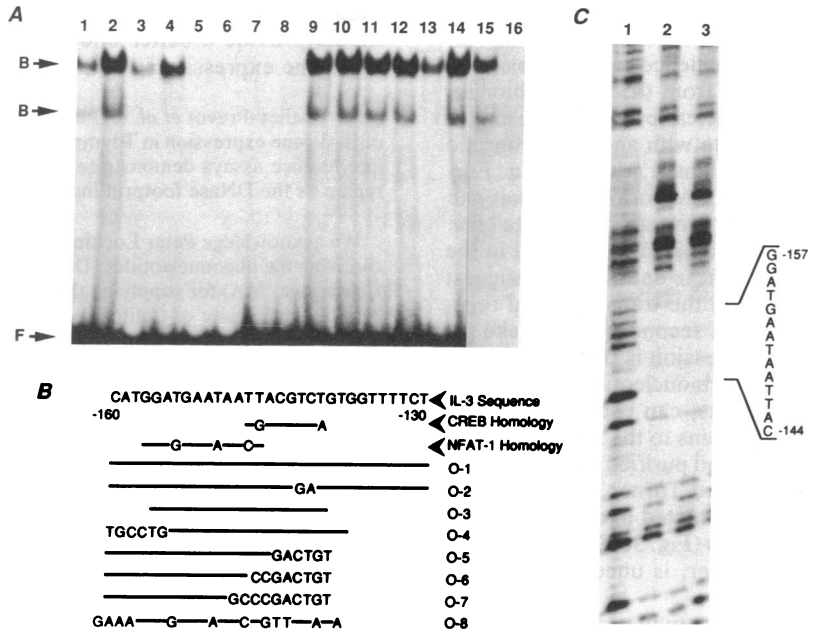

FIG. 3. DNA binding protein assays of the 161/126 region of IL-3. (*A*) Mobility-shift assays. Nuclear extracts from unstimulated Jurkat (lane 1), MLA (lane 3), HeLa (lane 5), or AML-193 (lane 6) cells, or Jurkat (lanes 2, 7–16) or MLA (lane 4) cells stimulated for 3 hr with Con A and PMA were incubated with an excess of unlabeled irrelevant duplex oligonucleotide (CGTCAGATAAAGATCCTTCCGA, lanes 1–6), an oligonucleotide (O–1) containing the 160/130 sequence (lane 7), a mutant CREB site sequence (lane 8, O-2), the 308/276 sequence of the IL-3 gene (lane 9, CAGTGAGCTGAGTCAGGCTTCCCCTTCCTGCCACG), the AP-1-containing region of the metallothionein promoter (ref. 26; lane 10, GAGCCGCAAGTGACTCAGCGCGGGGCGTGTGCAGG), the sequence used to clone a CREB cDNA (ref. 12; lane 11, GATCCGGCTGACGTCATCAAGCTA), the sequence used to clone a CRE-BP-1 cDNA (ref. 15; lane 12, TCGAGCTCCTAGCCTGACGTCAGAGAGAGAGC), the NFIL-2A sequence (refs. 22 and 27; lane 13, AGTCTTTGAAAATATGTGTAATATG), the NFAT-1 sequence of the IL-2 enhancer (lane 14, O-8), or with either of two mutant IL-3 5′ flanking sequences (lanes 15 and 16, O-4 and O-5). The bound (bands B) and free (bands F) oligonucleotides are noted. (*B*) The sequence of the IL-3 5′ flanking region from positions −160 to −130 is shown, along with the CREB and NFAT-1 homologies and the oligonucleotide sequences used in mobility-shift assays (identical nucleotides are underlined and mismatched positions are indicated by the altered base). (*C*) DNase I footprinting analysis. The results from the sense strand are presented, identical results were obtained using the antisense strand. Lanes: 1, no extract; 2, control MLA cell extract; 3, Con A/PMA-stimulated MLA cell extract.

failed to eliminate the enhancement of reporter gene activity detected in this region. (*ii*) Oligonucleotides that contain the CREB or the NFAT-1 binding sites were unable to displace specific binding to this IL-3 gene sequence and did not produce similar bands when tested in mobility-shift assays (data not shown). (*iii*) By using Northern blot analysis, no specific CREB transcripts could be detected in stimulated Jurkat or MLA cell RNA. In addition to binding nuclear proteins, this site is functionally active as its mutation eliminated CAT activity in reporter gene studies. Based on the results of mobility-shift competition assays and DNase I footprinting analysis, we propose the cognate binding site GATGAATAAT. Thus these findings argue strongly for the presence of a transcriptional activating protein involved in the regulation of IL-3 gene expression in T lymphocytes, here termed NF-IL3-A. The low-affinity competition shown by the NF-IL2-A site, a region that contains sequence similarity to the immunoglobulin octomer site, is intriguing (22, 27) and suggests that NF-IL3-A may be related to the octomer binding proteins.

A second region involved in the transcriptional regulation of IL-3 maps to an AP-1 site located at position −301. This sequence binds a family of proteins that includes the protooncogenes c-*jun* and c-*fos* and the related genes *JUN-B* and *JUN-D* (11, 14). Several lines of evidence point to the

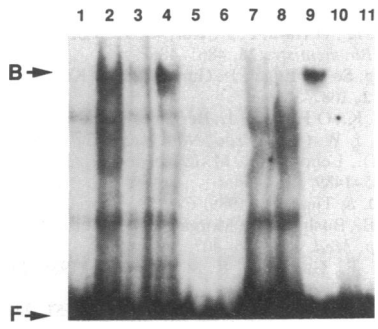

FIG. 4. Mobility-shift assay of the 308/276 region of IL-3. Nuclear extracts from unstimulated Jurkat (lane 1), MLA (lane 3), HeLa (lane 5), or AML-193 (lane 6) cells or from Jurkat (lanes 2, 7, and 8) or MLA (lane 4) cells stimulated for 3 hr with Con A and PMA, or purified recombinant c-jun protein (lanes 9–11) was incubated with an excess of unlabeled irrelevant duplex oligonucleotide (lanes 1–6 and 9), with oligonucleotide containing the 308/276 sequence (lanes 7 and 10), or with the AP-1-containing region of the metallothionein promoter (lanes 8 and 11). Bands: B, bound oligonucleotides; F, free oligonucleotides.

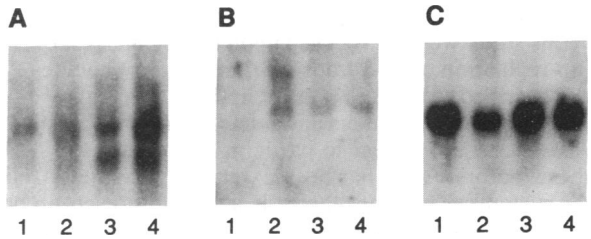

FIG. 5. Northern blot analysis of MLA-cell transcription factors. Poly(A) RNA (5 μg) from unstimulated MLA cells (lane 1) or cells stimulated with Con A and PMA for 15 min (lane 2), 2 hr (lane 3), or 6 hr (lane 4) was hybridized with cDNA probes for murine c-jun (*A*) or for bovine β-actin (*C*) or with a genomic probe for human *FOS* (*B*).

involvement of AP-1 proteins in the transcriptional regulation of IL-3 in lymphocytes. Mobility-shift assays showed that an oligonucleotide derived from the sequence between positions −306 and −276, and one derived from the metallothionein promoter AP-1 site, both bind a nuclear protein(s) present in T cells, cross-compete, and migrate with an electrophoretic mobility identical to that of recombinant c-jun protein. Furthermore, we demonstrated that RNA from both Jurkat and MLA cells contains c-jun and c-fos transcripts. Muegge *et al.* (28) have also shown that AP-1 proteins are involved in the regulation of IL-2 transcription. These data strongly suggest that AP-1 proteins are involved in the transcriptional regulation of IL-3 in human T cells. A second potential site for AP-1 regulation of IL-3 gene expression is present between positions −147 and −140. This octanucleotide matches the cognate CREB site at six positions, can compete with low affinity for binding of nuclear proteins to the AP-1 site of the IL-3 gene (data not shown), can bind purified c-jun protein in DNase footprinting assays (Richard Turner, personal communication), and appears to be protected in our footprinting assays using T-cell nuclear extracts (Fig. 3*C*), The functional significance of this region, however, is uncertain and warrants further study.

The presence of these binding activities in unstimulated cells, albeit at a lower level than in stimulated T-lymphocyte nuclear extracts, does not necessarily militate against a role for these proteins in the transcriptional activation of the IL-3 gene. Additional alterations, such as protein phosphorylation or enhanced binding affinity, not detectable by mobility-shift or DNase footprinting assays may be responsible for transcriptional activation (27, 29, 30).

Finally, despite the presence of a number of consensus sequences for characterized DNA binding proteins, the first 126 bp of the IL-3 gene 5′ flanking region displays a minimal response in the presence of mitogenic stimulation. Two of these sequences, termed CK-1 and CK-2 by Shannon *et al.* (23), are present beginning at positions −126 and −115, respectively. A second CK-1 site is also present at position −333 of the IL-3 5′ flanking region. In addition, the decanucleotide sequence beginning at position −74 matches the consensus transcription factor AP-2 binding site proposed by Imagawa *et al.* (24) at all 10 positions, and the decanucleotide sequences beginning at positions −96 and −90 match this consensus sequence at 8 positions. Comparison of the reporter gene activities of constructs that flank the CK-1 and CK-2 site, constructs 126 vs. 116 and constructs 116 vs. 107, respectively, or of the constructs that flank the consensus AP-2 sites, constructs 107 vs. 59, revealed minimal modulation of CAT activity across these sites in the 5′ flanking region of the IL-3 gene. Thus, the functional significance of the CK-1, CK-2, and AP-2 sites cannot be determined from their presence alone. It is possible that these elements require additional upstream sequences to exert an influence on transcription. Additional deletion mutations of these regions will be required to determine whether CK-1, CK-2, and AP-2 are functionally involved in the regulation of IL-3 gene expression in T lymphocytes.

In this study we have focused upon the regions responsible for up-regulation of IL-3 transcription in T lymphocytes. We have described sequences present in the 5′ flanking region of the IL-3 gene that act in cis and nuclear proteins that bind to these regions and appear to participate in this process. One of these factors, AP-1, has been extensively characterized in other systems. The other factor is a protein present in both control and mitogen-stimulated T cells. A more complete characterization of this putative transcriptional regulator awaits studies in nonlymphoid cell systems, *in vivo* crosslinking of nuclear proteins to transfected DNA fragments, and, of course, its genomic cloning. Along with further characterization of the down-modulating elements, these studies should provide a better understanding of the regulation of IL-3 gene expression in human T lymphocytes.

Note. Mathey-Prevot *et al.* (31) have also reported on the regulation of IL-3 gene expression in T lymphocytes. In this study methylation-interference assays demonstrate protein–DNA contact in the same region as the DNase footprinting experiments shown in Fig. 3*C*.

We acknowledge Peter Lockhart (Zymogenetics, Seattle) for synthesizing the oligonucleotides, Dr. Steven Clark (Genetics Institute, Cambridge, MA) for supplying the gibbon IL-3 cDNA, Dr. Richard Turner (University of California, San Francisco) for supplying the recombinant c-jun protein and the cDNA sequence for human *JUN-B*, Dr. Terry Meyer (Massachusetts General Hospital, Boston) for supplying the CREB cDNA, and Dr. William Lamph (Salk Institute, La Jolla, CA) for supplying the c-jun cDNA. This work was supported by National Institutes of Health Grants CA 31615 and 5 T32 HL 07093. K.K. is the recipient of a Junior Faculty Research Award of the American Cancer Society.

1. Lopez, A. F., To, L. B., Yang, Y.-C., Gamble, J. R., Shannon, M. F., Burns, G. F., Dyson, P. G., Juttner, C. A., Clark, S. C. & Vadas, M. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2761–2765.
2. Messner, H. A., Yamasaki, K., Jamal, N., Minden, M. M., Yang, Y.-C., Wong, G. G. & Clark, S. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6765–6769.
3. Kimoto, M., Kindler, V., Higaki, M., Ody, C., Izui, S. & Vassalli, P. (1988) *J. Immunol.* **140**, 1889–1894.
4. Kobayashi, M., Van Leeuwen, B. H., Elsbury, S., Martinson, M. E., Young, I. G. & Hapel, A. J. (1989) *Blood* **73**, 1836–1841.
5. Rothenberg, M. E., Owen, W. F., Silberstein, D. S., Woods, J., Soberman, R. J., Austen, K. F. & Stevens, R. L. (1988) *J. Clin. Invest.* **81**, 1986–1992.
6. Valent, P., Schmidt, G., Bessemer, J., Mayer, P., Zenke, G., Liehl, E., Hinterberger, W., Lechner, K., Maurer, D. & Bettelheim, P. (1989) *Blood* **73**, 1763–1769.
7. Ernst, T. J., Ritchie, A. R., Stopak, K. S. & Griffin, J. D. (1989) *Blood* **74**, Suppl. 1, 116.
8. Niemeyer, C. M., Sieff, C. A., Mathey-Prevot, B., Wimperis, J. Z., Bierer, B. E., Clark, S. C. & Nathan, D. G. (1989) *Blood* **73**, 945–951.
9. Kaushansky, K. (1989) *J. Immunol.* **143**, 2525–2529.
10. Yang, Y.-C., Ciarletta, A. B., Temple, P. A., Chung, M. P., Kovacic, S., Witek-Giannotti, J. S., Leary, A. C., Kriz, R., Donahue, R. E., Wong, G. G. & Clark, S. C. (1986) *Cell* **47**, 3–10.
11. Lamph, W. W., Wamsley, P., Sassone-Corsi, P. & Verma, I. M. (1988) *Nature (London)* **334**, 629–631.
12. Hoeffler, J. P., Meyer, T. E., Yun, Y., Jameson, J. L. & Habener, J. F. (1988) *Science* **242**, 1430–1433.
13. Degen, J. L., Neubauer, M. G., Degen, S. J. F., Seyfried, C. E. & Morris, D. R. (1983) *J. Biol. Chem.* **258**, 12153–12162.
14. Curren, T., McConnell, W. P., van Straaten, F. & Verma, I. M. (1983) *Mol. Cell. Biol.* **3**, 914–921.
15. Maekawa, T., Sakura, H., Kanei-Ishii, C., Sudo, T., Yoshimura, T., Fujisawa, J., Yoshida, M. & Ishii, S. (1989) *EMBO J.* **8**, 2023–2028.
16. Wood, W. I., Gitschier, J., Lasky, L. A. & Lawn, R. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1585–1588.
17. Kaushansky, K., O'Hara, P. J., Hart, C. E., Forstrom, J. W. & Hagen, F. S. (1987) *Biochemistry* **26**, 4861–4867.
18. Ponte, P., Ng, S.-Y., Engel, J., Gunning, P. & Kedes, L. (1984) *Nucleic Acids Res.* **12**, 1687–1696.
19. Kaushansky, K., O'Hara, P. J., Berkner, K., Segal, G. M., Hagen, F. S. & Adamson, J. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3101–3105.
20. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 1475–1489.
21. Bohmann, D. & Tjian, R. (1989) *Cell* **59**, 709–717.
22. Durand, D. B., Bush, M. R., Morgan, J. G., Weiss, A. & Crabtree, G. R. (1987) *J. Exp. Med.* **165**, 395–407.
23. Shannon, M. F., Gamble, J. R. & Vadas, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 674–678.
24. Imagawa, M., Chiu, R. & Karin, M. (1987) *Cell* **51**, 251–260.
25. Yang, Y.-C. & Clark, S. C. (1987) *Lymphokines* **15**, 375–387.
26. Turner, R. & Tjian, R. (1989) *Science* **243**, 1689–1694.
27. Crabtree, G. R. (1989) *Science* **243**, 355–361.
28. Muegge, K., Williams, T. M., Kant, J., Karin, M., Chiu, R., Schmidt, A., Siebenlist, U., Young, H. A. & Durum, S. K. (1989) *Science* **246**, 249–251.
29. Chiu, R., Boyle, W. J., Meek, J., Smeal, T., Hunter, T. & Karin, M. (1988) *Cell* **54**, 541–552.
30. Montmimy, M. R. & Bilezikjian, L. M. (1987) *Nature (London)* **328**, 175–178.
31. Mathey-Prevot, B., Andrews, N. C., Murphy, H. S., Kreissman, S. G. & Nathan, D. G. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5046–5050.