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# Cloning of a cellular factor, interleukin binding factor, that binds to NFAT-like motifs in the human immunodeficiency virus long terminal repeat

**(fork head gene)**

CHING LI*, CHINGFENG LAI*, DAVID S. SIGMAN*†, AND RICHARD B. GAYNOR*‡

Division of Hematology-Oncology, Departments of *Medicine and †Biological Chemistry, University of California, Los Angeles, School of Medicine and Molecular Biology Institute, Los Angeles, CA 90024

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ABSTRACT **Human immunodeficiency virus (HIV) gene expression is regulated by both general transcription factors and factors induced by activation of T lymphocytes such as NF-κB and the nuclear factor of activated T cells (NFAT). Within the HIV long terminal repeat (LTR), two purine-rich domains between nucleotides −283 and −195 have homology to a regulatory region found in the interleukin 2 promoter, which binds NFAT and other cellular factors. In the HIV LTR, this region has been demonstrated to have both positive and negative regulatory effects on HIV gene expression. In an attempt to clone genes encoding cellular factors that bind to these NFAT-like elements in the HIV LTR, we used λgt11 expression cloning with oligonucleotides corresponding to these binding motifs. A ubiquitously expressed cDNA encoding a 60-kDa protein, which we termed interleukin binding factor (ILF), binds specifically to these purine-rich motifs in the HIV LTR. This factor also binds to similar purine-rich motifs in the interleukin 2 promoter, though with lower affinity than to HIV LTR sequences. Sequence analysis reveals that the DNA binding domain of ILF has strong homology to the recently described fork head DNA binding domain found in the *Drosophila* homeotic protein fork head and a family of hepatocyte nuclear factors, HNF-3. Other domains found in ILF include a nucleotide binding site, an N-glycosylation motif, a signal for ubiquitin-mediated degradation, and a potential nuclear localization signal. These results describe a DNA binding protein that may be involved in both positive and negative regulation of important viral and cellular promoter elements.**

The regulation of the human immunodeficiency virus (HIV) gene expression is dependent on a variety of cellular transcription factors and the viral transactivator protein tat. A number of elements in the HIV long terminal repeat (LTR), including SP1, TATA, and TAR are involved in regulating gene expression in a variety of cell lines (1). At least two other regulatory regions are important for activation of HIV LTR gene expression in activated T lymphocytes. One is the enhancer region extending from nucleotides −103 to −78, which contains two NF-κB motifs (2–4). The other is a region extending from nucleotides −283 to −195, which contains several motifs with homology to a critical regulatory domain in the interleukin-2 (IL-2) promoter (5–7). This motif in the IL-2 promoter is a positive-acting element that binds a cellular factor known as the nuclear factor of activated T cells (NFAT), whose binding is strongly induced in activated T cells (5–7). In the HIV LTR, this region containing NFAT-like motifs is important for increasing HIV gene expression in activated T cells, but it has little effect on basal gene expression (8). However, in the presence of tat it appears to have negative regulatory effects on HIV gene expression (8, 9). Thus, it may bind different cellular factors that are involved in both positive and negative control of gene expression.

DNase I footprinting demonstrates the binding of cellular factors from −254 to −216 in the HIV LTR with extracts prepared from activated but not resting T cells (6, 7). The binding of cellular factors to this protected region is blocked by competition with a −285 to −255 fragment containing the NFAT binding site in the IL-2 promoter, suggesting that NFAT binds to the HIV LTR (6, 7). An increase in NFAT binding precedes the activation of both IL-2 and HIV gene expression in stimulated T cells (6, 7). The induction of NFAT binding requires new protein synthesis (6, 7), and its binding is inhibited by cyclosporin, an inhibitor of the enzyme cyclophilin (10). These results are consistent with a model in which NFAT binding is augmented in activated T cells by several potential mechanisms, including by increases in its synthesis, by posttranslational modifications of itself or other factors, and by decreases in the activity of specific inhibitory proteins. However, the production of NFAT is not completely restricted to activated T cells since low levels of NFAT binding are also detected in resting T cells (11).

A variety of cellular proteins bind to NFAT-like regulatory motifs (12). In activated T cells, UV crosslinking demonstrates that three cellular proteins of 90, 45, and 25 kDa bind to the NFAT motif in the IL-2 promoter (12). Cyclosporin inhibits the binding of each of these proteins, indicating that multiple proteins with a similar pattern of regulation may bind to this region. Recently, a cellular factor restricted to B lymphocytes and macrophages, known as PU.1 (13, 14), with strong homology to the *ets* protooncogene was found to bind to a related motif found in simian virus 40 (15). Thus, different DNA binding proteins are likely capable of binding to these purine-rich motifs.

In an attempt to identify cellular factors that interact with similar regulatory motifs in the HIV LTR and IL-2 promoter, we used the technique of λgt11 expression cloning with oligonucleotides corresponding to these motifs (16). We isolated a ubiquitous cellular factor that bound to both of these motifs, though with different binding affinities. A cDNA encoding a 60-kDa protein termed interleukin binding factor (ILF)[§] was isolated, and its DNA binding domain was found to be homologous to the recently described fork head DNA binding domain (17). This factor is a candidate for a

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Abbreviations: ILF, interleukin binding factor; NFAT, nuclear factor of activated T cells; IL-2, interleukin 2; HNF-3, hepatocyte nuclear factor; HIV, human immunodeficiency virus; LTR, long terminal repeat.

‡To whom reprint requests should be addressed at: Division of Molecular Virology, Department of Internal Medicine, Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, TX 75235.

§The nucleotide sequence of the ILF cDNA has been deposited in the GenBank data base (accession no. X60787).

cellular factor that appears to be involved in the regulation of gene expression of important viral and cellular promoters.

## MATERIALS AND METHODS

**Identification of the ILF Gene.** The ILF gene was identified by screening HeLa, B cell, and Jurkat λgt11 cDNA libraries (obtained commercially from Clontech) with $^{32}$P-labeled wild-type and mutant double-stranded ligated oligonucleotide probes as described (17). The sequence of the wild-type oligonucleotides correspond to a portion of the HIV LTR (3) extending either from −283 to −256 (distal ILF motif; 5′-GAAGAGGCCAATGAAGGAGAGAACAACA-3′) or −223 to −195 (proximal ILF motif; 5′-GAGGACGCG-GAGAAAGAAGTGTTAGTGTG-3′). The sequence of the mutant oligonucleotide for the distal ILF motif was 5′-GTCGTGGCCTCTGTCTAGTGTGGCAACA-3′. Positive phage were subsequently purified and screened in tertiary platings. A 706-base-pair fragment containing the ILF DNA binding domain isolated from a HeLa cDNA library was labeled by random priming and used to screen a human HeLa cDNA λZAP library (Stratagene) to identify full-length cDNA clones. DNA sequence analysis of these clones employed the Sanger method with the Sequenase system (United States Biochemical).

**Construction of Glutathione *S*-Transferase–ILF Fusion Proteins.** A 1.3-kilobase (kb) *Xma* I/*Eco*RV fragment extending from nucleotide 807 to nucleotide 2174 in the ILF cDNA was first cloned into the same sites of the Bluescript vector (18). The recombinant plasmid was digested with *Bam*HI and *Eco*RV for in-frame subcloning into the *Bam*HI and *Sma* I sites of pGEX-3X (Pharmacia) (19) to express a glutathione *S*-transferase–ILF fusion protein of 72 kDa (ILFΔ1). ILFΔ2 was constructed by insertion of an *Xma* I/*Sfi* I fragment extending from nucleotide 807 to nucleotide 1620 into pGEX-3X, giving a 58-kDa fusion protein. ILFΔ3 was constructed by inserting an *Xma* I/*Eco*RI fragment extending from nucleotide 807 to nucleotide 1075 into pGEX-3X, yielding a 37-kDa fusion protein.

**Gel Retardation Analysis.** Double-stranded oligonucleotides used in gel retardation analysis (20) correspond to the two ILF motifs in the HIV LTR between −283 and −256 (distal) and between −223 and −195 (proximal) (3), the NFAT motif in the IL-2 promoter between −285 and −254 (6), a transcription factor AP1 binding site in the adenovirus early region 3 promoter between −103 and −83 (20), and a transcription factor CREB binding site in the somatostatin promoter extending from −60 and −33 (21). The sequences are as follows: 5′-AATTGGAGGAAAAACTGTTTCATA-CAGAAGGCGT-3′ (NFAT motif in the IL-2 promoter), 5′-GGTTCCTCCTTGGCTGACGTCAGAGAGAGA-3′ (CREB binding site), 5′-GAAGTTCAGATGACTAAC-TCA-3′ (AP1 binding site). A 56-nucleotide *Alu* I fragment extending from −310 to −255 in the HIV LTR was also isolated and end-labeled for use in gel retardation assays. β-Galactosidase–ILF fusions were prepared as described (18) and purified by using monoclonal anti-β-galactosidase chromatography (Promega). Glutathione *S*-transferase fusion proteins were purified with affinity chromatography as described (20). Fusion proteins were detected with rabbit polyclonal antibody raised to trypE–ILF fusion proteins and monoclonal anti-β-galactosidase antibody by using Western blot analysis.

## RESULTS

**Isolation of cDNA Clones Encoding ILF.** To clone genes encoding cellular factors that bind to purine-rich motifs in the HIV LTR, we used modifications of the λgt11 expression cloning procedure (16). The homology of these sequences to related purine-rich regions found in other viral and cellular promoters discussed in this study are shown in Table 1. Oligonucleotides extending from either −283 to −256 or −223 to −195 in the HIV LTR referred to hereafter as the distal and proximal ILF motifs, respectively, were ligated and used to probe λgt11 cDNA libraries prepared from either HeLa or lymphoid (B-cell or Jurkat) RNA. Several isolates were identified from each of these libraries that specifically bound to both of the ILF motifs but not to mutated oligonucleotides (data not shown). Each of these isolates contained regions of identical amino acids homologous to the so-called fork head DNA binding domain (17). One of these cDNAs, ILF, isolated from a λgt11 HeLa cDNA library was further characterized.

Table 1. Related sequence motifs in HIV and IL-2 promoters

| Promoter | Sequence | |
|---|---|---|
| IL-2 | −291 AGAAAGGAGGAAAAA | −277 |
| HIV, distal | −276 CCAATGAAGGAGAGA | −262 |
| HIV, proximal | −220 GACGCGGAGAAAGAA | −206 |
| MHC | −70 CCAAGTGAGGAACCA | −56 |
| SV40 PU box | −319 CTGAAAGAGGAACTT | −305 |

Homologous purine-rich sequences in the IL-2 promoter (5), major histocompatibility complex (MHC) class II promoter (14), the HIV LTR (3), and the simian virus 40 (SV40) PU box (14) are illustrated.

**ILF Binds to Related Sequences in the HIV LTR and IL-2 Promoter.** To characterize the β-galactosidase–ILF fusion protein produced from the λgt11 HeLa cDNA isolate, lysates were prepared from phage-infected *Escherichia coli* and purified by anti-β-galactosidase-Sepharose chromatography (Fig. 1*A*, lanes 3 and 4). In addition, β-galactosidase produced from λgt11 phage not containing a cDNA insert was purified by anti-β-galactosidase-Sepharose chromatography and used as a control (Fig. 1*A*, lanes 1 and 2). As shown in Fig. 1, the β-galactosidase protein migrated at ≈110 kDa, whereas the β-galactosidase–ILF fusion protein migrated at 135 kDa (Fig. 1*A*, lanes 2 and 4). Some breakdown of the β-galactosidase occurred in these samples resulting in several lower molecular mass species of between 70 and 90 kDa. To further characterize these proteins, Western analysis was performed with antibody generated to either β-galactosidase (Fig. 1*B*) or ILF (Fig. 1*C*). As shown in Fig. 1*B*, both β-galactosidase and β-galactosidase–ILF were reactive with anti-β-galactosidase (lanes 1 and 2), whereas only the β-galactosidase–ILF fusion protein was detected with anti-ILF (Fig. 1*C*, lanes 1 and 2).

Gel retardation analysis with either the β-galactosidase protein or the β-galactosidase–ILF fusion protein was then performed with an end-labeled HIV LTR fragment extending from −310 to −255 (Fig. 2). As shown in Fig. 2, the β-galactosidase–ILF fusion protein bound to the HIV LTR probe (lane 3), whereas the β-galactosidase protein did not bind under identical conditions (lane 2). Competition analysis was performed to demonstrate the specificity of this binding. Oligonucleotides corresponding to either the distal ILF motif in the HIV LTR (Fig. 2, lanes 4 and 5) or the NFAT motif in the IL-2 promoter (Fig. 2, lanes 6 and 7) specifically blocked by competition the binding of the β-galactosidase–ILF protein. Likewise, the proximal ILF motif in the HIV LTR also resulted in complete competition of ILF protein binding (data not shown). However, we note that in a number of different experiments both the proximal and distal HIV motifs served as better competitors for ILF binding than the NFAT motif. Oligonucleotides corresponding to either AP1 (Fig. 2, lanes 8 and 9) or CREB (Fig. 2, lanes 10 and 11) binding sites did not compete for the binding of ILF. These results demonstrated that ILF binds specifically to related purine-rich sequences in the HIV LTR and IL-2 promoter.

**ILF Contains a Fork Head DNA Binding Domain.** A fragment containing the ILF DNA binding domain was used as a

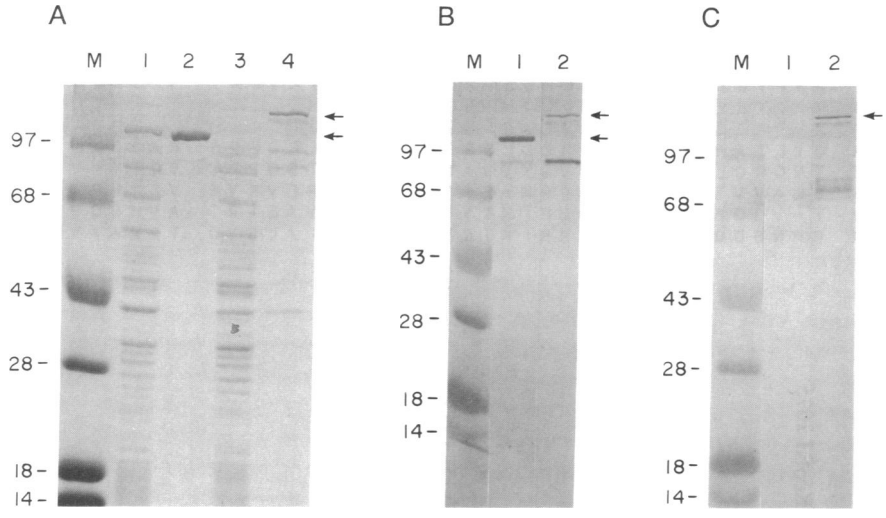


FIG. 1. Production of β-galactosidase fusion proteins from λgt11 isolates. (*A*) Lysates were prepared from *E. coli* infected with λgt11 (lanes 1 and 2) or from a λgt11 isolate containing a portion of the ILF cDNA (lanes 3 and 4). β-Galactosidase (lane 2) and the β-galactosidase–ILF fusion protein (lane 4) were purified using anti-β-galactosidase-Sepharose chromatography. The samples were electrophoresed on SDS/10% polyacrylamide gels and Coomassie stained. Western blot analysis of affinity-purified samples was performed with antibody directed against either β-galactosidase (*B*) or ILF (*C*). Molecular mass markers (in kDa) are given at the left. (*B* and *C*) Lane 1, β-galactosidase; lane 2, β-galactosidase–ILF fusion protein; lane M, molecular mass markers.

probe to attempt to isolate full-length cDNAs from a HeLa cDNA library. A 3.0-kb cDNA was isolated, and the sequence of this clone between nucleotides 929 and 1634 was identical to that of the probe. RNase T2 protection studies of either HeLa or Jurkat mRNA using different portions of the 3.0-kb cDNA as probes demonstrated the integrity of this clone, which was also confirmed by Southern blot analysis (data not shown). An open reading frame of 543 amino acids, which extended from a potential initiating methionine at nucleotide 518 to a stop codon at nucleotide 2147, was identified. *In vitro* translation of ILF RNA in rabbit reticulocyte lysate yielded a 60-kDa species (data not shown). There was a stop codon 138 nucleotides upstream of the methionine residue, and the homology to a consensus Kozak sequence suggested that an initiating methionine was present (22). The amino acid sequence of this open reading frame and a restriction map of the cDNA are shown in Fig. 3.

Comparison of the amino acid sequence of ILF with those from GenBank (August, 1990) revealed a number of interesting homologies. There was strong homology of a 98-amino-acid region of ILF with both the *Drosophila* regulatory protein fork head (23) and the DNA binding domain of the hepatocyte specific factor (HNF-3A) (24). Within this region of ILF and fork head, there was 46% amino acid identity and 81% homology, allowing for conservative amino acid changes. The region of homology between ILF, fork head, and HNF-3A is shown in Fig. 4. (17) A number of other conserved amino acid motifs were also noted (Fig. 4). These include a potential nuclear localization signal (RKRRPR) (25), a sequence (GAGSSG) with homology to a nucleotide binding site found in a number of enzymes including the *ras* oncogene (26), a potential ubiquitin-mediated degradation signal (RTPLGPLSS) (27), and a potential N-glycosylation site (NGT). Finally, it was noted that ILF contained a high content of several amino acids, including proline (11%), serine (9.6%), and threonine (7.7%). Thus, ILF contains amino acid motifs consistent with other cellular transcription factors.

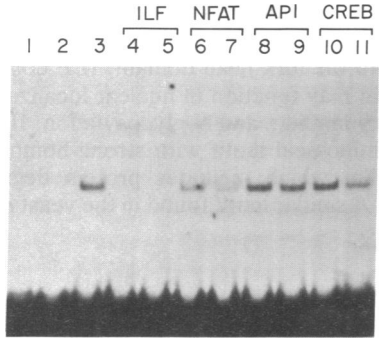


FIG. 2. Gel retardation analysis with the β-galactosidase–ILF fusion protein. An HIV LTR fragment extending from −310 to −255 was used in gel retardation assays with either β-galactosidase or β-galactosidase–ILF fusion protein purified with anti-β-galactosidase-Sepharose chromatography. Lane 1, probe alone; lane 2, probe with 3 μg of β-galactosidase extract; lane 3, probe with 3 μg of β-galactosidase–ILF extract. Competition analysis with either a 10-fold (lanes 4, 6, 8, 10) or 50-fold (lanes 5, 7, 9, 11) molar excess of unlabeled oligonucleotides corresponding to ILF (lanes 4 and 5), NFAT (lanes 6 and 7), AP1 (lanes 8 and 9), or CREB (lanes 10 and 11) binding motifs is shown.

**Deletion of the ILF Fork Head Domain Eliminates its DNA Binding.** To determine the region of the ILF protein that was required for DNA binding, deletions were placed into the ILF cDNA. These ILF deletion constructs were fused to the glutathione *S*-transferase gene in the bacterial expression plasmid pGEX-3X (19). The pGEX expression system was used to facilitate the purification of glutathione *S*-transferase fusion proteins with glutathione-agarose affinity chromatography. The constructs tested included a deletion of the amino terminus of ILF, a deletion of both the amino and carboxyl termini of ILF, and a deletion of the fork head domain in addition to the amino and carboxyl termini of ILF. Oligonucleotides corresponding to the distal ILF site in the HIV LTR, the NFAT site in the IL-2 promoter, and an HIV LTR fragment containing the distal ILF site were used in gel retardation analyses.

There was no binding of the purified glutathione *S*-transferase protein to any of these probes (Fig. 5, lane 1). Deletion of portions of both the amino and the carboxyl termini of ILF resulted in a protein that retained specific binding to all these probes (Fig. 5, lane 2). Deletion of the fork head binding domain in addition to the amino and carboxyl termini of ILF resulted in a protein that did not bind to any of these probes (Fig. 5, lane 3). A deletion of the amino terminus of ILF resulted in specific binding to all three probes (Fig. 5, lane 4). The binding specificity of each of these proteins was similar using the proximal ILF binding site (data not shown). These results implicate the fork head domain in the DNA binding properties of ILF to the HIV LTR and the IL-2 promoter.

## DISCUSSION

The nucleotide sequence of the HIV LTR reveals several purine-rich motifs between nucleotides −283 and −195 with homology to an element in the IL-2 promoter (5). This latter element is a binding site for the cellular factor NFAT, whose binding is induced in activated T cells (5–7). In an attempt to clone genes that encode cellular factors capable of binding to these motifs in

A

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  1  MAQVFVDGVF QRRGAPPLQL PRVCTFRFPS TNIKITFTAL SSEKREKQEA SESPVKAVQP
 61  HISPLTINIP DTMAHLISPL PSPTGTISAA NSCPSSPRGA GSSGYKVGRV MPSDLNLMAD
121  NSQPENEKEA SGGDSPKDDS KPPYSYAQLI VQAITMAPDK QLTLNGIYTH ITKNYPYYRT
181  ADKGWQNSIR HNLSLNRYFI KVPRSQEEPG KGSFWRIDPA SESKLIEQAF RKRRPRGVPC
241  FRTPLGPLSS RSAPASPNHA GVLSAHSSGA QTPESLSREG SPAPLEPEPG AAQPKLAVIQ
301  EARFAQSAPG SPLSSQPVLI TVQRQLPQAI KPVTYTVATP VTTSTSQPPV VQTVHVVHQI
361  PAVSVTSVAG LAPANTYTVS GQAVVTPAAV LAPPKAEAQE NGDHREVKVK VEPIPAIGHA
421  TLGTASRIIQ TAQTTPVQTV TIVQQAPLGQ HQLPIKTVTQ NGTHVASVPT AVHGQVNNAA
481  ASPLHMLATH ASASASLPTK RHNGDQPEQP ELKRIKTEDG EGIVIALSVD TPPAAVREKG
541  VQNZ
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B

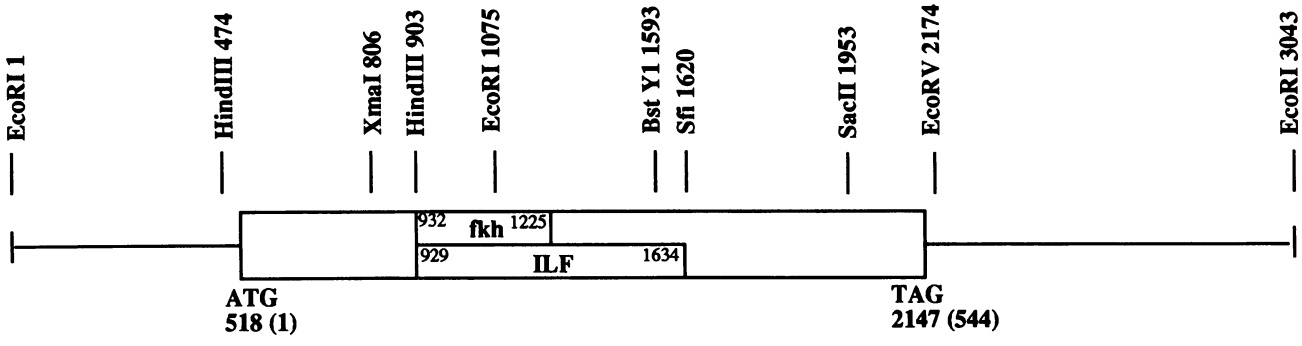


FIG. 3. Amino acid sequence of the ILF coding region. (*A*) A 543-amino-acid open reading frame encoded by a portion of the ILF cDNA is shown. The shaded region indicates the region of homology of ILF with the fork head and HNF-3A DNA binding domains. The circled region (GAGSSG) contains homology with a nucleotide binding sequence, the boxed region (RKRRPR) contains a potential nuclear localization signal, the underlined sequence (RTPLGPLSS) is homologous to a region required for ubiquitin-mediated degradation, and the dashed box (NGT) indicates a potential N-glycosylation site. (*B*) The 3.0-kb ILF cDNA is shown and the position of the 1629-base-pair open reading frame is indicated. The nucleotides (929–1634) in the original ILF cDNA and the region of homology with the fork head (fkh) DNA binding domain (nucleotides 932–1225) are indicated. The positions of restriction sites in the ILF cDNA are also indicated.

the HIV LTR, λgt11 expression cloning with ligated DNA binding motifs was used. A cellular factor known as ILF was identified, which binds to these purine-rich motifs in both the HIV LTR and the IL-2 promoter. Northern blotting analysis reveals that this factor is constitutively expressed in both lymphoid and nonlymphoid cells. This suggests that ILF is a ubiquitous cellular factor that binds to similar purine-rich motifs. Preliminary results suggest that ILF is a transcriptional repressor with negative regulatory properties for both the HIV LTR and the IL-2 promoters.

Analysis of the ILF gene reveals that it is capable of giving rise to a number of different gene products by alternative splicing. However, the DNA binding domain of each of these species has strong homology with a 98-amino-acid region found in both the *Drosophila* fork head protein (23) and in the gene family of hepatic nuclear factors known as HNF-3 (24). The *Drosophila* fork head gene is involved in the regulation of terminal development in the *Drosophila* embryo (23), whereas the HNF-3 gene family is predominantly expressed in cells that derive from the lining of the primitive gut (28). The binding sites of the fork head protein to *Drosophila* promoters have not yet been defined, whereas members of the HNF-3A family have been demonstrated to bind and activate gene expression from important regulatory elements of the transthyretin and α-1 antitrypsin genes, which are required for hepatocyte-specific gene expression (29). The sequences to which HNF-3A binds do not have a high degree of homology to the purine-rich sequences found in the HIV LTR and IL-2 promoter. Further binding studies and methylation interference will be required to determine if the highly homologous fork head domain may bind to divergent recognition sequences.

In addition to the fork head domain, ILF contains amino acid motifs that may function in nuclear localization, nucleotide specificity binding, and N-glycosylation. ILF also contains a nine-amino-acid motif with strong homology to that found in cyclin, which mediates protein degradation by ubiquitin (27). A similar motif found in the yeast α2 repressor

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ILF      DSKPPYSYAQLIVQAITMAPDKQLTLNGIYTHITKNYPY
         ••xxxxxx xx• xx •x•• xxx••xx x • •x•
FKH      HAKPPYSYISLITMAIQNNPTRMLTLSEIYQFIMDLFPF
         xxxxxxxxxxxxxxxxxx •x••xxxxxxxxxx•xxxxx•
HNF-3A   HAKPPYSYISLITMAIQQAPSKMLTLSEIYQWIMDLFPY

ILF        YRTADKGWQNSIRHNLSLNRYFIKVPRSQEEPGKGSFW
           xx ••• xxxxxxx•xx•x •x•x•xx••• xxxxxxx
FKH        YRQNQQRWQNSIRHSLSFNDCFVKIPRTPDKPGKGSFW
           xxxxxxxxxxxxxxxxxxx xxxx• x•xxxxxxxx•x
HNF-3A     YRQNQQRWQNSIRHSLSFNACFVKVARSPDKPGKGSYW

ILF        RIDPASESKLIEQAFRKRRPR
           ••x•x•••• • • •x• x
FKH        TLHPDSGNMFENGCYLRRQKR
           xxxxxxxxxxxxxxxxxxxxx
HNF-3A     TLHPDSGNMFENGCYLRRQKR
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FIG. 4. Homology of the ILF DNA binding domain. The amino acid sequence of a portion of the ILF DNA binding domain (amino acids 138–236) is aligned with the DNA binding domains of the *Drosophila* fork head protein (FKH) (amino acids 208–306) and the hepatocyte factor HNF-3A (amino acids 168–266). The x indicates amino acid identity, and the • indicates a conservative amino acid change.

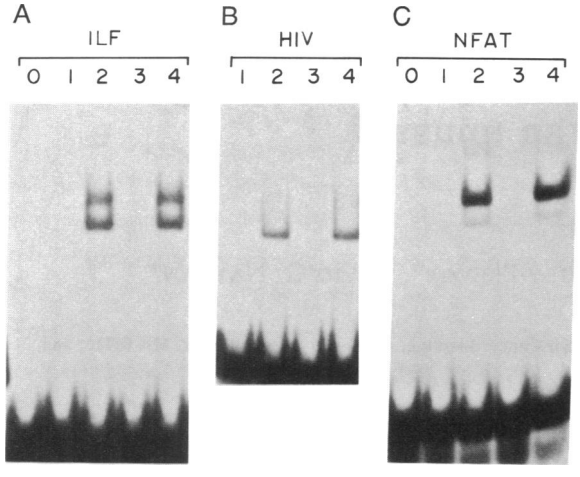


FIG. 5. Gel retardation analysis of glutathione *S*-transferase–ILF fusions. Gel retardation analysis was performed with either oligonucleotides extending from −283 to −256 in the HIV LTR (ILF) (*A*), a fragment extending from −310 and −255 in the HIV LTR (HIV) (*B*), or oligonucleotides extending from −285 to −254 in the IL-2 promoter (NFAT) (*C*) and 4 μg of each of the glutathione *S*-transferase–ILF fusions. Lane 0, probe alone; lane 1, glutathione *S*-transferase protein alone; lane 2, a deletion of both the amino and carboxyl termini in the glutathione *S*-transferase–ILF fusion (ΔILF3); lane 3, a deletion of the ILF fork head DNA binding domain in addition to the amino and carboxyl termini in the glutathione *S*-transferase–ILF fusion (ΔILF2); lane 4, an amino-terminal deletion of ILF in the glutathione *S*-transferase–ILF fusion (ΔILF1).

may mediate the *in vivo* degradation of this protein (30). Thus, it is possible that the activation of T lymphocytes results in the degradation of ILF protein followed by subsequent binding of other cellular proteins such as NFAT to these purine-rich motifs, with resultant increases in gene expression.

Multiple cellular proteins have been demonstrated to bind to the NFAT motifs in the IL-2 promoter (12). The binding of NFAT is strongly induced by T-cell activation, and this increase in NFAT binding precedes the activation of both HIV and IL-2 gene expression in stimulated T cells (6, 7). This suggests a role for NFAT in the T-cell activation of both of these genes (6, 7). Whether ILF or any related genes that we have isolated are related to NFAT will require a detailed comparison of these proteins with purified NFAT protein. A macrophage- and B-cell-specific transcription factor, PU.1, with homology to the *ets* oncogene, also binds to a purine-rich sequence (GAGGAA) identical to that found in the NFAT motif (14). This protein has been demonstrated to have positive effects on gene expression (15). Thus, it is likely that multiple genes may encode factors that bind to these similar regulatory motifs. A study of these different factors will be required to determine how they may regulate gene expression in both a positive and negative manner.

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