LEF-1, a gene encoding a lymphoid-specific with protein, an HMG domain, regulates T-cell receptor α enhancer function

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Lymphoid-specific cDNA clones were isolated that encode a nuclear protein with homology to the chromosomal nonhistone protein HMG-1 and to putative regulators of cell specialization, including the mammalian testis-determining factor SRY and fungal mating-type proteins. The gene represented by the isolated cDNA clones, termed LEF-1 (lymphoid enhancer-binding factor 1), is developmentally regulated and expressed in pre-B and T lymphocytes but not in later-stage B cells or nonlymphoid tissues. Both endogenous and recombinant LEF-1 were shown to bind to a functionally important site in the T-cell antigen receptor (TCR) α enhancer. Maximal TCRα enhancer activity was found to parallel the cell type-specific expression pattern of LEF-1. Moreover, forced expression of recombinant LEF-1 in late stage B cells increases TCRα enhancer function. Taken together, these data suggest that LEF-1 is a regulatory participant in lymphocyte gene expression and differentiation.

[*Key Words*: HMG box protein; TCRα enhancer; transcriptional control; lymphoid-specific gene expression]

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Lymphocyte differentiation converts progenitor cells into highly specialized cells of either the B- or the T-cell lineage. Both cell lineages involve multiple stages of differentiation that have been characterized and defined by changes in the expression patterns of their antigen receptor genes. Early-stage B lymphocytes, termed pre-B cells, express and rearrange their immunoglobulin heavy-chain (IgH) gene. Further differentiation, involving rearrangement of the light-chain locus, yields B cells that express membrane-bound immunoglobulin on their surface. Upon exposure to antigen, B cells ultimately mature into antibody-secreting plasma cells (for review, see Blackwell and Alt 1988; Cooper and Burrows 1989). Likewise, differentiating T cells undergo stage-specific changes in the expression of genes encoding the T-cell antigen receptor (TCR) and accessory molecules. Early-stage T cells undergo sequential rearrangement of their TCRβ- and TCRα-chain genes and express both CD4 and CD8 surface molecules. These cells differentiate into mature T cells expressing both TCR chains, and either the CD4 or the CD8 coreceptor molecule (for review, see Davis and Bjorkman 1988; Blackman et al. 1990).

To gain insight into some of the mechanisms underlying terminal differentiation, several studies were aimed at identifying the *cis*- and *trans*-acting regulatory components of genes specific for the differentiated phenotypes. Expression of immunoglobulin genes in B cells and TCR genes in T cells was shown previously to depend on transcriptional enhancers located 5′ or 3′ of their respective constant regions (Gillies et al. 1983; Banerji et al. 1983; Grosschedl and Baltimore 1985; Krimpenfort et al. 1988; McDougall et al. 1988; Ho and Leiden 1989; Winoto and Baltimore 1989). These enhancers function specifically in lymphocytes. The IgH enhancer is active in all B cells and in a subset of T cells (Gerster et al. 1986). In contrast, the TCRβ-chain enhancer is functional in T cells and in early-stage B cells (Krimpenfort et al. 1988; Takeda et al. 1990). Both the immunoglobulin and TCR enhancers were subsequently used to identify and purify nuclear factors that bind to specific nucleotide sequences within the enhancers. Some of these factor-binding sites were used to isolate complementary DNAs (cDNAs) of genes encoding enhancer-binding proteins (Murre et al. 1989; Henthorn et al. 1990; Ho et al. 1990; Roman et al. 1990). Surprisingly, most of the nuclear factors and genes identified by these approaches are ubiquitously expressed. To date, one gene, termed Oct-2, was isolated and shown to encode a lymphocyte-specific protein that binds to the OCTA site in both the IgH enhancer and promoter (Muller et al. 1988; Scheidereit et al. 1988; Staudt et al. 1988). The OCTA site, however, can also be recognized by other

related proteins such as Oct-1, which is expressed in virtually all cell types (Singh et al. 1986; Scholer et al. 1989).

On the basis of the notion that the binding of ubiquitous factors may obscure the identification of cell type-specific factors binding the same site, we employed an alternative strategy for the cloning of genes encoding putative lymphocyte-specific regulatory factors. We first generated a set of lymphocyte-specific cDNA clones. Binding of the encoded polypeptides to DNA was then used as a criterion to identify cDNA clones that encode putative regulatory proteins. Lymphocyte specificity is thus ensured prior to screening for the ability to bind DNA.

Here, we report the molecular cloning of cDNAs of a developmentally regulated murine gene that is expressed specifically in pre-B and T lymphocytes. This gene, termed LEF-1 (for lymphoid enhancer-binding factor 1), encodes a nuclear protein that binds to a functionally important site in the TCRα enhancer and confers maximal activity. LEF-1 is a new member of a family of regulatory proteins that share homology with the high mobility group protein 1 (HMG-1). We propose LEF-1 as a putative regulatory participant in lymphocyte differentiation.

Results

Cloning of cDNAs encoding LEF-1

To identify candidate transcriptional regulators of the lymphocyte lineage, we isolated several lymphoid-specific cDNA clones from the murine pre-B-cell line 70Z/3 by differential screening of recombinant bacteriophages with radiolabeled first-strand cDNA probes from either 70Z/3 or murine erythroleukemia (MEL) poly(A)$^+$ RNA (see Materials and methods). To examine polypeptides encoded by these cDNA clones for their ability to bind to DNA–cellulose, the inserts from eight different recombinant bacteriophages were subcloned into vectors containing an ATG in each of the three reading frames, transcribed with T7 RNA polymerase, and the transcripts were translated in a reticulocyte lysate in the presence of [^{35}S]methionine. One lymphoid-specific cDNA clone, termed GL1, yielded a protein that bound to DNA–cellulose, remained bound at 150 mM KCl, and could be eluted at 250 mM KCl (data not shown). The nucleotide sequence of the GL1 cDNA was determined and found to contain an open reading frame fused to an upstream ATG in the vector. A longer cDNA clone, termed GN8, was isolated, which extended an additional 1 kb at the 5′ end of the GL1 open reading frame (Fig. 1a). Analysis of the nucleotide sequence of the GN8 cDNA revealed a single open reading frame of 397 amino acids, beginning with an ATG at nucleotide 990 and terminating with a stop codon at nucleotide 2181 (Fig. 1b). The open reading frame is preceded by a long untranslated region with three stop codons in the same frame, suggesting that GN8 contains the entire coding sequence. Conceptual translation of the open reading frame predicts a protein with a molecular mass of 44 kD. On the basis of its lymphoid-specific expression pattern and specific binding to transcriptional enhancer sequences (see below), we term the GN8-encoded protein LEF-1.

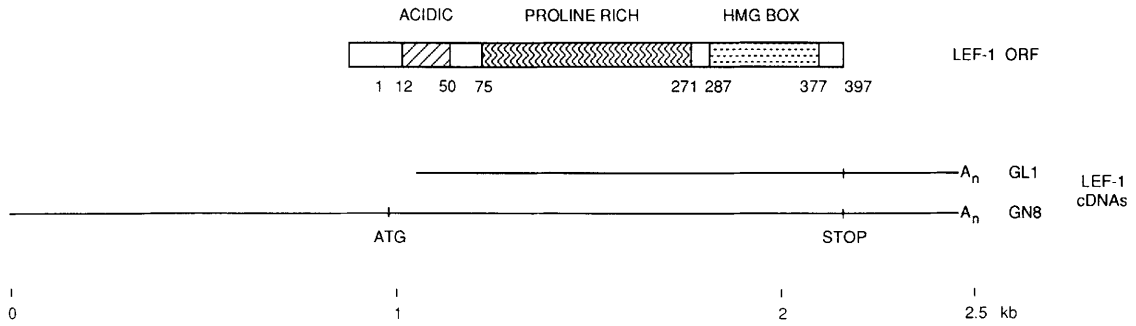
LEF-1 is an HMG box protein

The predicted amino acid sequence of LEF-1 has structural features suggestive of a transcriptional regulator. Amino acid sequence comparison of LEF-1 with sequences in the Protein Identification Resource/NBRF revealed a region of homology to the nonhistone chromosomal protein HMG1 (Wen et al. 1989) and the mating type protein Mat Mc of *Schizosaccharomyces pombe* (Kelly et al. 1988). This region of LEF-1, comprising ~85 amino acids and designated the HMG box (Fig. 1a,b), was also found to share amino acid sequence homology with the human upstream binding factor (hUBF), a nucleolar protein that binds and transcriptionally activates the rRNA gene promoter (Jantzen et al. 1990). hUBF contains four HMG boxes, one of which appears to be sufficient for DNA binding (Jantzen et al. 1990). More recently, additional members of this new family of proteins have been identified. A gene from the sex-determining region of the human and mouse Y chromosome, termed SRY, encodes a testis-specific HMG box protein that has been proposed to play a crucial role in testis development (Gubbay et al. 1990; Sinclair et al. 1990). Likewise, the genetically defined regulatory gene *mt a1*, which is involved in mating type specification of *Neurospora crassa*, encodes an HMG box protein (Staben and Yanofsky 1990).

Alignment of the HMG boxes of LEF-1 and other members of the family (Fig. 2) revealed 25% identity and 47% similarity with the Mc mating-type protein of *S. pombe* (Kelly et al. 1988) and 25% identity and 41% similarity with murine SRY (Gubbay et al. 1990; for the percentage of amino acid identity and similarity with other HMG boxes and for allowed conservative amino acid substitutions, see the legend to Fig. 2). With the exception of one amino acid gaps in Mat Mc and mt a1, no spacing changes were necessary for the optimal alignment of the HMG boxes. The HMG box of LEF-1 appears to be more closely related to those of the fungal mating-type proteins and SRY than to that of hUBF and HMG-1. The homology of LEF-1 with the various members of the HMG box proteins is concentrated in three regions within the HMG box: a block of eight neutral and hydrophobic amino acids flanked by charged residues from position 10 to 26, a region containing conserved hydrophobic and basic residues between positions 41 and 78, and a basic region at the carboxyl terminus that is most noticeable in LEF-1 (Fig. 2). We also note that the HMG box of LEF-1 differs from those of the other members of the family in the region from position 33 to 40, where amino acid conservation is exclusive of LEF-1 (Fig. 2).

The predicted amino acid sequence of LEF-1 also includes a segment of 197 amino acids containing 37 proline residues and a 39-amino-acid region containing 14 aspartic and glutamic acid residues (Fig. 1a). Proline-rich

a



b

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1    CGGAGGAGCCGAGGGGCCACCGGCAGCTCTTTGCTTTGACCGAGGGGAGGCGGGGAGAGAGGCGAGCCAGGCAGAAACTCGAGCCGGGAACAAAGAGGGATCGGGCTCGAGTGTGTGTG

120  TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTAAGTGTGTGTATCGGCCCGAGCTGCGGGCTGGAACATTTGGGCCCGAAGCTCCTGCTGTGACTCCCCAAGACTCCGCCGTGCCAGC

239  CACCGCCGATTCCCAGCGCTCATCATCACAAACTTTATTCTTGGCAAACTTCTCTTTTTCTCCCCTCCCCCTCCAGCAGATTAAATGCTCCTCCAGAAGGAAAACCGAAGCGAAAGGGA

358  AGGAAAGAAGCTCTAACGCGGACGTCTGCAGCCCGGTGGCTCTTTATTGTTTACTCTGAAGGAAGTGGACTTTTCGGTATTTTCTGATTCTTCTCGTACCTCCGCTGGGGCAAAGGGAG

477  CCTCTTGGCCAGCTCTCCTCTTCTCAAAAACAAAACAAAACAAAACCCCAAATCACCGAAATCAGCATCCGAGGAATCCGTGGAGAGGTTTTGCACGGTGACGCACCACGGGACCCTCG

596  TTTGCACTTTGGCAAGTCGTGTCCCCTCCGGAACCGGTGTCCTCGAAGCACAGACCCCAGCAACGAGTGCGGCGTGGGGTGGCCACCCAGCCCTGGCAGCCTAGCCTAGTGCACGCGGA

715  GCGCGTAGACGCTCTCAGAGGCGCCGGCGTGCCGCCCTCGCAGTCTGGTGCCCTTCGCCCCAGGCCGGGGCGCCGGCGTCCTTCTAAGTGGGAAAGCGCGGCAATCGCAGAGGCTCCTG

834  CAGCTGCTCCAGCCGTGGGCCCCTCGCTTGCCCACCCGGCTTCCATTCACAGTCCTCCCGCACTCAGTCTTCCAACTCCCCCACCTCACATCGACCCTCCCTTCTTGCTGTCTCGGAGT

                                          1                                  10                                  20
                                         Met Pro Gln Leu Ser Gly Gly Gly Gly Gly Gly Asp Pro Glu Leu Cys Ala Thr Asp Glu
953  GCTCCCGCAGCGGAGCGGAGATTACACAGCCGCCGGG   ATG CCC CAA CTT TCC GGA GGA GGC GGC GGG GGG GAC CCG GAA CTC TGC GCC ACC GAT GAG

                                     30                                  40                                  50
     Met Ile Pro Phe Lys Asp Glu Gly Asp Pro Gln Lys Glu Lys Ile Phe Ala Glu Ile Ser His Pro Glu Glu Glu Gly Asp Leu Ala Asp
1050 ATG ATC CCC TTC AAG GAC GAA GGC GAT CCC CAG AAG GAG AAG ATC TTC GCC GAG ATC AGT CAT CCC GAA GAG GAG GGC GAC TTA GCC GAC

                                     60                                  70                                  80
     Ile Lys Ser Ser Leu Val Asn Glu Ser Glu Ile Ile Pro Ala Ser Asn Gly His Glu Val Val Arg Gln Ala Pro Ser Ser Gln Glu Pro
1140 ATC AAG TCA TCT TTG GTT AAC GAG TCC GAA ATC ATC CCA GCC AGC AAC GGG CAT GAG GTG GTC AGA CAA GCC CCG TCC TCT CAG GAG CCC

                                     90                                  100                                 110
     Tyr His Asp Lys Ala Arg Glu His Pro Asp Glu Gly Lys His Pro Asp Gly Gly Leu Tyr Asn Lys Gly Pro Ser Tyr Ser Ser Tyr Ser
1230 TAC CAC GAC AAG GCC AGA GAA CAC CCT GAT GAA GGA AAG CAT CCA GAC GGA GGC CTG TAC AAC AAG GGA CCC TCC TAC TCC AGT TAC TCT

                                    120                                 130                                 140
     Gly Tyr Ile Met Met Pro Asn Met Asn Ser Asp Pro Tyr Met Ser Asn Gly Ser Leu Ser Pro Pro Ile Pro Arg Thr Ser Asn Lys Val
1320 GGC TAC ATA ATG ATG CCC AAT ATG AAC AGC GAC CCG TAC ATG TCA AAT GGG TCC CTT TCT CCA CCC ATC CCG AGG ACA TCA AAT AAA GTG

                                    150                                 160                                 170
     Pro Val Val Gln Pro Ser His Ala Val His Pro Leu Thr Pro Leu Ile Thr Tyr Ser Asp Glu His Phe Ser Pro Gly Ser His Pro Ser
1410 CCC GTG GTG CAG CCC TCT CAC GCG GTC CAC CCG CTC ACC CCC CTC ATC ACC TAC AGC GAC GAG CAC TTT TCT CCG GGA TCC CAC CCG TCA

                                    180                                 190                                 200
     His Ile Pro Ser Asp Val Asn Ser Lys Gln Gly Met Ser Arg His Pro Pro Ala Pro Glu Ile Pro Thr Phe Tyr Pro Leu Ser Pro Gly
1500 CAC ATC CCG TCA GAT GTC AAC TCC AAG CAA GGC ATG TCC AGA CAC CCT CCA GCT CCT GAA ATC CCC ACC TTC TAC CCC CTG TCT CCG GGC

                                    210                                 220                                 230
     Gly Val Gly Gln Ile Thr Pro Pro Ile Gly Trp Gln Gly Gln Pro Val Tyr Pro Ile Thr Gly Gly Phe Arg Gln Pro Tyr Pro Ser Ser
1590 GGC GTT GGA CAG ATC ACC CCA CCC ATT GGC TGG CAA GGT CAG CCT GTT TAT CCC ATC ACG GGT GGA TTC AGG CAA CCC TAC CCA TCC TCA

                                    240                                 250                                 260
     Leu Ser Gly Asp Thr Ser Met Ser Arg Phe Ser His His Met Ile Pro Gly Pro Pro Gly Pro His Thr Thr Gly Ile Pro His Pro Ala
1680 CTG TCA GGC GAC ACT TCC ATG TCC AGG TTT TCC CAT CAT ATG ATT CCT GGT CCC CCT GGC CCC CAC ACA ACT GGC ATC CCT CAT CCA GCT

                                    270                                 280                                 290
     Ile Val Thr Pro Gln Val Lys Gln Glu His Pro His Thr Asp Ser Asp Leu Met His Val Lys Pro Gln His Glu Gln Arg Lys Glu Gln
1770 ATT GTA ACA CCT CAG GTC AAA CAG GAG CAC CCC CAC ACG GAC AGT GAC CTA ATG CAC GTG AAG CCT CAA CAC GAA CAG AGA AAG GAG CAG

                                    300                                 310                                 320
     Glu Pro Lys Arg Pro His Ile Lys Lys Pro Leu Asn Ala Phe Met Leu Tyr Met Lys Glu Met Arg Ala Asn Val Val Ala Glu Cys Thr
1860 GAG CCC AAA AGA CCT CAT ATT AAG AAG CCT CTG AAT GCT TTC ATG TTA TAT ATG AAA GAA ATG AGA GCG AAT GTC GTA GCT GAG TGC ACG

                                    330                                 340                                 350
     Leu Lys Glu Ser Ala Ala Ile Asn Gln Ile Leu Gly Arg Arg Trp His Ala Leu Ser Arg Glu Glu Gln Ala Lys Tyr Tyr Glu Leu Ala
1950 CTA AAG GAG AGT GCA GCT ATC AAC CAG ATC CTG GGC AGA AGA TGG CAC GCC CTC TCC CGG GAA GAG CAG GCC AAA TAC TAT GAA CTA GCA

                                    360                                 370                                 380
     Arg Lys Glu Arg Gln Leu His Met Gln Leu Tyr Pro Gly Trp Ser Ala Arg Asp Asn Tyr Gly Lys Lys Lys Lys Arg Lys Arg Glu Lys
2040 CGG AAA GAG AGA CAG CTA CAC ATG CAG CTT TAT CCA GGC TGG TCA GCG CGA GAC AAT TAT GGC AAG AAG AAG AAG AGG AAG AGA GAG AAG

                                    390                 397
     Leu Gln Glu Ser Thr Ser Gly Thr Gly Pro Arg Met Thr Ala Ala Tyr Ile OP
2130 CTA CAG GAG TCG ACT TCA GGT ACA GGT CCC AGA ATG ACA GCT GCC TAC ATC TGA AACATGGTGGTAAGAGAAGCTCCTTCCCAACGTGCAAAGCCAAGGCAG

2232 CGACCCCAGGCCCTCTTCTGGAGATGGAAGCTTGTTGAAACCCCAGACTGTCTCCACAGCTTGCCCGGCTGACCCCAAGGAACACTGACAGCAACCTTACCCTGAGGTCACTGCTAGCGC

2352 TGACCCGAAGACACAGTCACTGCCACCTCTTCCTTCTGTCGTCTACTGCAAGCGCCGACTTCCAAAAAGAAAGCCGAAAACGGTTGTTTCGGAAAAAAAAAAAAAAAAAA
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HMG box

Figure 1. (*See facing page for legend.*)

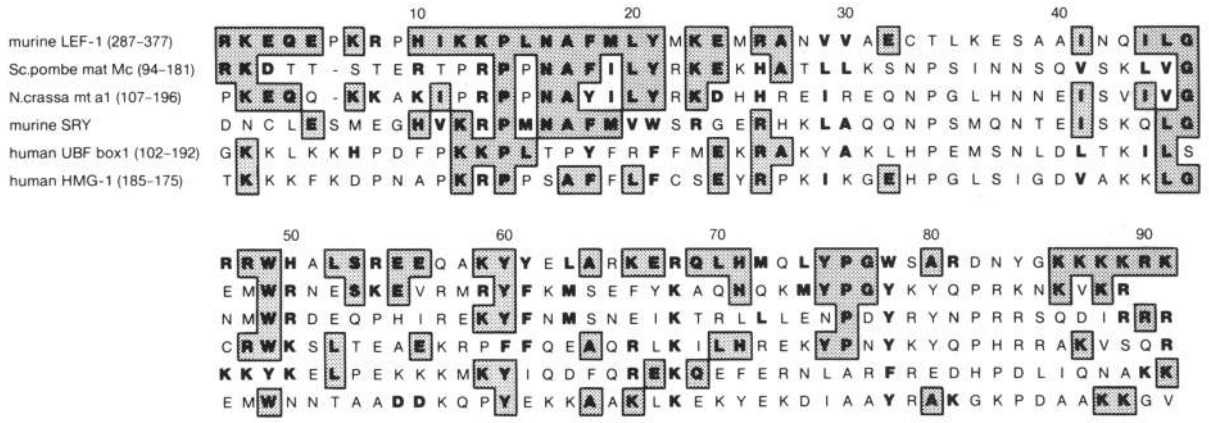

Figure 2. Amino acid sequence alignment of the HMG boxes of LEF-1 and other members of the family. The first line shows the HMG box of murine LEF-1. The amino acid positions of the HMG box in each protein are indicated in parentheses. The numbers above the amino acid sequence of LEF-1 indicate the relative positions within the HMG box. The HMG boxes of the mating-type proteins Mat Mc of *S. pombe* (Kelly et al. 1988) and mt a1 of *N. crassa* (Staben and Yanofsky 1990) show 25% and 22% amino acid sequence identity with the HMG box of LEF-1, respectively, and 47% and 41% sequence similarity. For optimal sequence alignment, one-amino-acid gaps were allowed in Mat Mc between positions 98 and 99, and in mt a1 between positions 111 and 112. The sequence identity and similarity of the HMG box of LEF-1 with that of the murine SRY (Gubbay et al. 1990) are 25% and 41%; with that of human hUBF (Jantzen et al. 1990) are 18% and 35%; and with that of human HMG-1 (Wen et al. 1989) are 21% and 32%. Amino acids identical among the various HMG boxes are boxed, and conserved amino acids are indicated in bold type. Conserved amino acid substitution groups are (K,R,H), (L,I,V), (W,Y,F), (E,D), and (L,M,I).

and acidic regions have been implicated previously in the activation functions of RNA polymerase II transcription factors (for review, see Mitchell and Tjian 1989).

Developmental expression pattern

To examine the expression pattern of LEF-1, poly(A$^+$) RNA from various mouse tissues was analyzed for the presence of transcripts hybridizing to a probe derived from the coding region of GN8 cDNA. RNA blot analysis revealed multiple-sized transcripts specifically in lymphoid tissues (lymph nodes, spleen, and thymus) and in testis (Fig. 3a). Two major transcripts of 2.7 and 4.2 kb were detected in thymus and at lower abundance in lymph nodes and spleen. Hybridization of the RNA blot with a DNA probe derived from the 5′ end of the GN8 cDNA revealed only the 4.2-kb major transcript, suggesting that the GN8 clone represents a partial cDNA of this transcript (data not shown). The 2.7-kb transcript appears to be generated by alternate promoter usage or alternate RNA processing, or may be derived from a very closely related gene. In addition, a series of low abundant transcripts ranging from 3.0 to 3.4 kb were detected in testis. Preliminary characterization of a clone isolated from a testis cDNA library using a GN8 cDNA probe indicates that these testis-specific transcripts are derived from the DNA strand opposite to that encoding the LEF-1 transcripts (Rong-guo Qiu and R. Grosschedl, unpubl.). Therefore, the LEF-1 gene appears to be expressed in a lymphoid-specific manner.

To gain further insight into the expression of LEF-1 within the lymphoid B- and T-cell lineages, RNA from murine and human cell lines representing various stages of differentiation was examined for the presence of LEF-1 transcripts (Fig. 3b). Two major LEF-1 transcripts of 4.2 and 2.7 kb and a minor LEF-1 transcript of 3.7 kb were detected in all pre-B-cell lines, whether derived from fetal liver (lanes 4–6) or from adult bone marrow (lanes 7–11). Maturation of the pre-B-cell lines PD31 and 70Z/3 with bacterial lipopolysaccharide (LPS; Nelson et al. 1984), resulted in a three- to sixfold decrease in the number of LEF-1 transcripts (cf. lane 8 with 9 and lane 10 with 11). Cell lines representing later stages of B-cell differentiation (mature B cells and plasmacytomas) did not contain any LEF-1 transcripts (lanes 12–17). However, LEF-1 transcripts were detected in all T-cell lines analyzed (lanes 18–22), irrespective of their differentiation stage. Finally, LEF-1 transcripts were not detected in any of the nonlymphoid cell lines examined (lanes 1–3), which include representatives of the myeloid (WEHI 3) and erythroid (MEL) hematopoetic lineages. Taken to-

Figure 1. Nucleotide sequence of the murine LEF-1 cDNA and predicted amino acid sequence of the encoded protein. (*a*) Schematic diagram of LEF-1 cDNA clones and the longest open reading frame. The two lines represent the cDNA clones GL1 and GN8. The open reading frame is shown above. (HMG box) The stippled box representing the region of homology with HMG-1. The hatched box depicts an acidic region (36% aspartic and glutamic acid); the zigzagged box represents a proline-rich region (19% proline). (*b*) Nucleotide sequence of the cDNA clone GN8 and predicted amino acid sequence of the encoded protein. The HMG homology is indicated by the box.

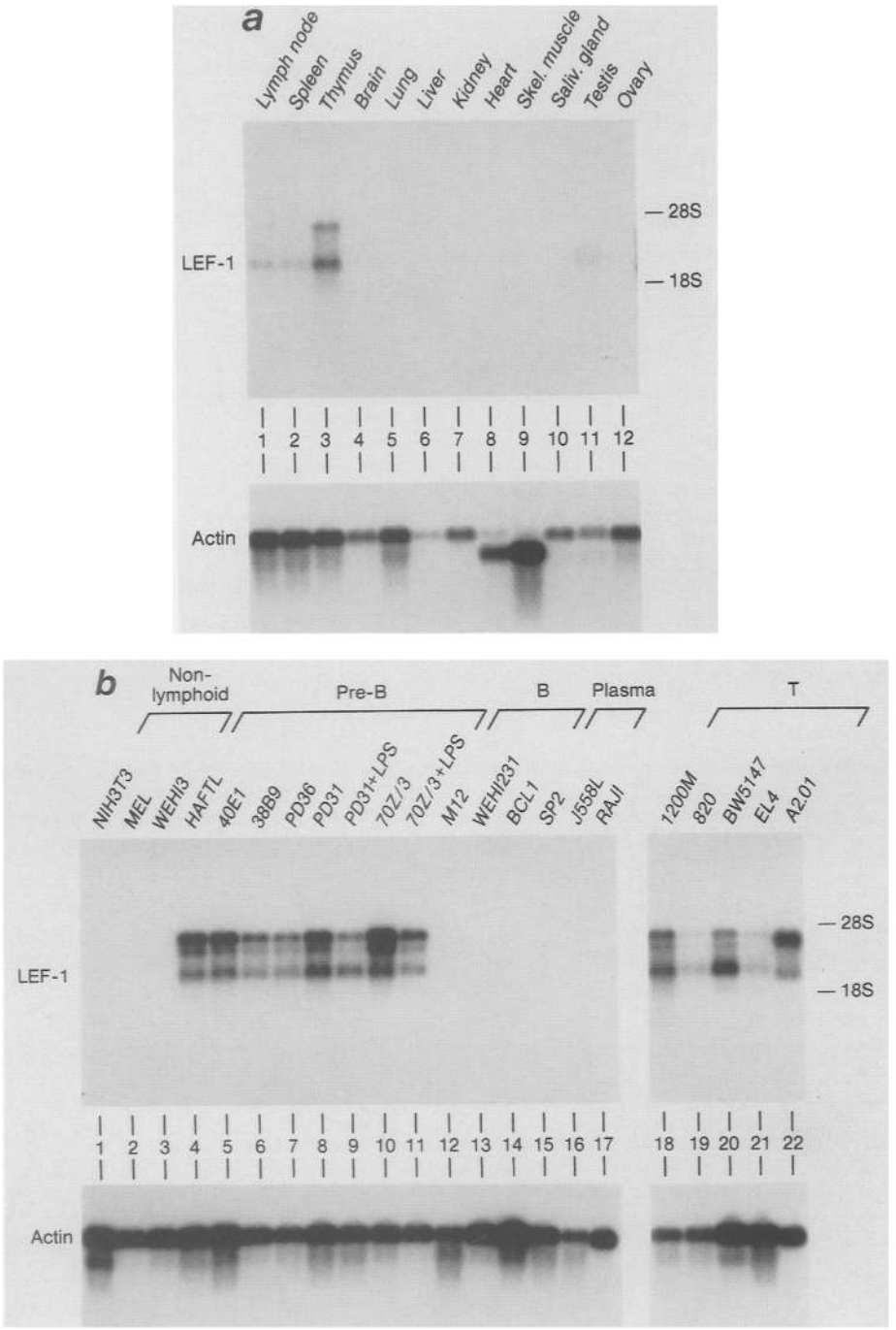

Figure 3. Expression of LEF-1 in mouse tissues and in mouse and human cell lines. (*a*) RNA blot analysis of LEF-1 transcripts in adult mouse tissues. Poly(A)$^+$ RNA (0.5 μg) from various tissues was size-fractionated by gel electrophoresis, transferred to a nylon membrane, and hybridized with a nick-translated DNA probe derived from the GN8 cDNA (nucleotides 1158–1517). The sizes of the major LEF-1 transcripts detected in thymus and at lower abundance in spleen and lymph nodes are ~2.7 and 4.2 kb, as determined by comparison with an 18S and 28S rRNA size marker. The transcripts detected in testis range in size from 3.0 to 3.4 kb. To control for RNA levels, a duplicate RNA blot was hybridized with a nick-translated hamster actin gene probe (*bottom*). (*b*) RNA blot analysis of LEF-1 transcripts in various cell lines. Poly(A)$^+$ RNA (0.5 μg) from various tissue culture cell lines was analyzed for the presence of LEF-1 transcripts as in *a*. RAJI and A2.01 are human lymphoid B- and T-cell lines, respectively. All other cell lines are derived from mouse. They include fetal liver-derived pre-B cells (HAFTL, 40E1, and 38B9), adult bone marrow-derived pre-B cells (PD36, PD31, and 70Z/3), B cells (M12, WEHI231, and BCL1), plasmacytomas (SP2 and J558L), and T cells, including three CD4$^-$/CD8$^-$ cell lines (BW5147, EL4, and A2.01), a CD4$^+$/CD8$^+$ cell line (820), and a CD4$^-$/CD8$^+$ cell line (1200M). Nonlymphoid cell lines include fibroblasts (NIH-3T3), erythroleukemia cells (MEL), and myeloid cells (WEHI 3). The sizes of the LEF-1 transcripts (4.2, 3.7, and 2.7) were estimated by comparison with an 18S and 28S RNA size standard.

gether, these observations suggest that LEF-1 is expressed specifically in pre-B and T lymphocytes and is conserved between mouse and man.

Detection and nuclear localization of endogenous LEF-1

To detect the endogenous LEF-1 protein in lymphoid cells, we raised rabbit polyclonal antibodies to a polypeptide derived from the GL1 cDNA by bacterial overexpression (see Materials and methods). This anti-LEF-1 serum was used to probe immunoblots of whole-cell extracts from various lymphoid and nonlymphoid cells (Fig. 4a, lanes 4–11). A major protein with an apparent molecular mass of 54 kD was detected in pre-B and T cells (lanes 5–8). Minor polypeptides of smaller apparent molecular mass were detected in T cells (lanes 5 and 6), but not in B-lineage cells or in nonlymphoid cells (lanes 4, 7–11). This suggests that the faster-migrating polypeptides represent alternate forms of LEF-1, degradation products, or other T-cell-specific proteins that can cross-react with the anti-LEF-1 serum. For comparison, the anti-LEF-1 serum was reacted with polypeptides that were translated in vitro from GL1- and GN8-derived transcripts (Fig. 4a, lanes 1–3). The GN8-derived polypeptide comigrated with the 54-kD protein detected in pre-B and T cells, supporting the conclusion that the GN8 cDNA contains the entire open reading frame of LEF-1.

The subcellular location of LEF-1 was examined by indirect immunofluorescence microscopy of 70Z/3 pre-B cells reacted with anti-LEF-1 serum (Fig. 4c). Although distinguishing lymphoid cell nuclei and cytoplasm is complicated by their small cytoplasmic volume, nuclear staining could be inferred by examining cells at high magnification (Fig. 4b) and by comparing the immunofluorescence staining of LEF-1 (Fig. 4c) with DAPI staining of DNA (Fig. 4d). LEF-1 staining was nuclear in nondividing cells, but it was distributed throughout the cytoplasm in dividing cells (Fig. 4b–d, cell pair in anaphase at lower right corner and cell in metaphase above it), consistent with their lack of a nuclear envelope. The level of background staining of the cells was determined with preimmune serum (Fig. 4e). These data indicate that LEF-1 is a nuclear protein.

Sequence-specific DNA binding

To determine whether LEF-1 binds DNA in a sequence-specific manner and to identify putative target sequences, we examined various lymphocyte-specific enhancers for LEF-1 binding. Our search was facilitated by two observations. First, activity of the TCRβ enhancer is observed in T and pre-B cells (Krimpenfort et al. 1988;

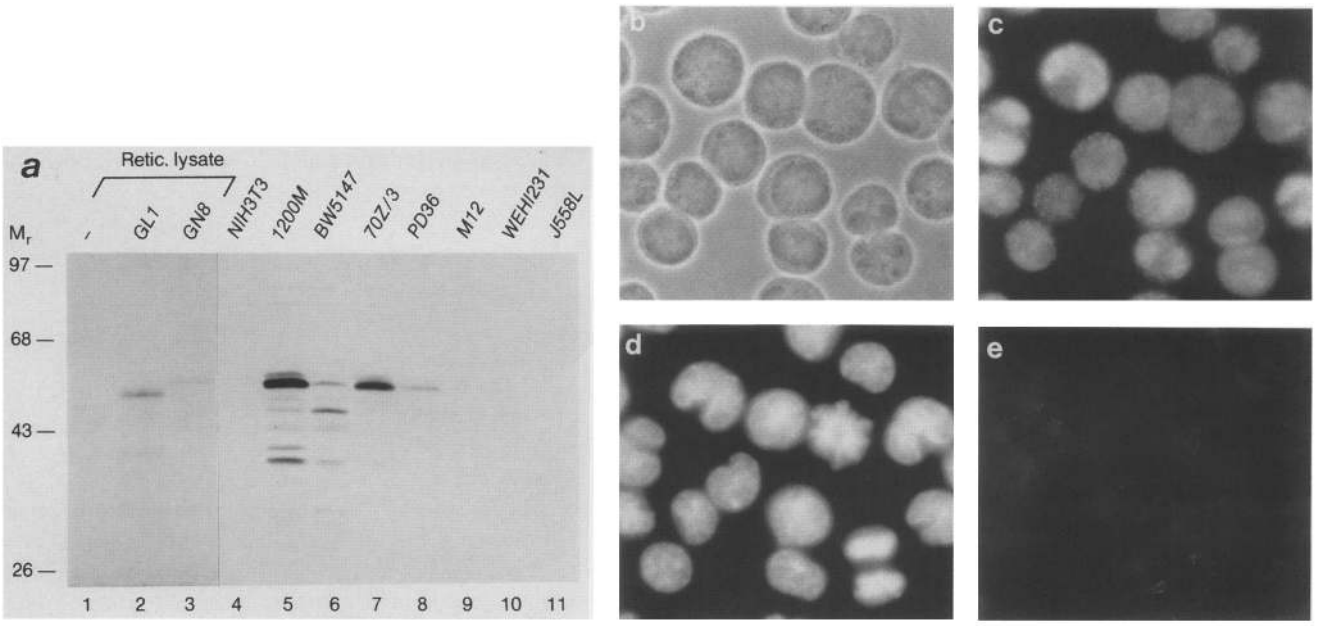

Figure 4. Protein identification and subcellular localization of LEF-1. (*a*) Immunoblot analysis of LEF-1 protein. In vitro-translated recombinant LEF-1 polypeptides (lanes *2* and *3*) and whole-cell protein extracts from various cell lines (lanes *4–11*) were size-fractionated by SDS-PAGE, transferred onto a nylon membrane, and reacted with precleared anti-LEF-1 serum (see Materials and methods) at a dilution of 1 : 3000. Recombinant LEF-1 polypeptides were translated in a reticulocyte lysate from GL1- or GN8-derived transcripts (see Materials and methods). The sizes of molecular mass markers are indicated in kD. (*b–e*) Immunocytochemical staining of endogenous LEF-1. Bright-field (*b*), rhodamine immunofluorescence (*c*), and 4,6-diamidino-2-phenylindole (DAPI) fluorescence (*d*) microscopy of the same field of fixed 70Z/3 pre-B cells reacted with precleared anti-LEF-1 serum at a dilution of 1 : 130 and double-stained with rhodamine-conjugated goat anti-rabbit antibodies and the DNA intercalating agent DAPI. (*e*) Immunofluorescence microscopy of fixed 70Z/3 cells incubated with preimmune serum at a 1 : 130 dilution.

Takeda et al. 1990), thus paralleling the expression pattern of LEF-1. Second, a DNA fragment comprising part of an enhancer associated with the murine CD4 gene can interact with a pre-B- and T-cell-specific nuclear factor in vitro (S. Sawada and D.R. Littman, pers. comm.). Electrophoretic mobility-shift and methylation interference assays performed with in vitro-translated LEF-1 protein indicated that LEF-1 can bind a specific sequence in this CD4 enhancer fragment (A. Amsterdam, S. Sawada, D.R. Littman, and R.Grosschedl, unpubl.). Nucleotide sequences closely related to this LEF-1-binding site were also found to be present in the human and mouse TCRα and TCRβ enhancers (Krimpenfort et al. 1988; McDougall et al. 1988a; Ho and Leiden 1989; Winoto and Baltimore 1989; Gottschalk and Leiden 1990; Takeda et al. 1990). Since the human TCRα enhancer has been well-characterized and delineated to a 116-bp DNA fragment, we selected this enhancer for studies of the binding specificity and the putative regulatory role of LEF-1.

For the studies aimed at examining DNA binding of LEF-1, a recombinant vaccinia virus was generated that allows for overexpression of LEF-1 in infected cells (see Materials and methods). Therefore, infection of HeLa cells, which lack endogenous LEF-1, with this recombinant vaccinia LEF-1 virus or a control vaccinia hemagglutinin (HA) virus enabled us to prepare nuclear extracts that differed only in the presence or absence of LEF-1. Immunoblot analysis of nuclear extracts of HeLa cells infected with the vaccinia LEF-1 virus indicated a level of LEF-1 five times higher than that detected in the T-cell line 1200M (data not shown).

Sequence-specific binding of recombinant LEF-1 to a minimal 98-bp TCRα enhancer fragment (TCRα98; Fig. 5a) was examined by DNase I footprinting with either HeLa/vaccinia LEF-1 or HeLa/vaccinia HA nuclear extracts (Fig 5b, lanes 3 and 4). A DNase I-protected region between nucleotides 62 and 76 was generated only with HeLa/vaccinia LEF-1 nuclear extract (lane 3), indicating sequence-specific binding of recombinant LEF-1 to the TCRα enhancer. Nucleotides flanking the LEF-1-binding site displayed an enhanced sensitivity to DNase I cleavage. Additional protection over a region from nucleotide 21 to 46 was observed with both HeLa cell nuclear extracts (lanes 3 and 4). This footprint coincides with the α_1 footprint that has been observed previously in T-cell nuclear extracts and covers a region containing a consensus cAMP-responsive element (CRE; Jones et al. 1988). The LEF-1-generated footprint coincides with a footprint generated by the recently identified and purified T-cell factor TCF-1α (Waterman and Jones 1990) and overlaps with a larger footprint that has been observed previously in T-cell nuclear extracts and has been termed α_2 (see Fig. 5a; Ho and Leiden 1989).

To gain further insight into LEF-1 binding, the methylation interference pattern of recombinant LEF-1 on a partially methylated 25-bp TCRα DNA fragment was determined using HeLa/vaccinia LEF-1 nuclear extract (Fig. 5a, TCRα25). Binding of LEF-1 is inhibited by methylation of G_{70} and partially inhibited by methylation of G_{69} (Fig. 5c). No methylated G nucleotides on the other strand interfered with LEF-1 binding (data not shown). On the basis of the methylation interference data, TCRα98 and TCRα25 oligonucleotides containing a triple point mutation in the LEF-1-binding site were generated for subsequent studies (Fig. 5a).

Sequence specificity of the LEF-1/TCRα25 protein–DNA interaction was demonstrated by competition of binding with wild-type and mutated TCRα25 oligonucleotides (Fig. 6a). The LEF-1/TCRα25 complex was detected in an electrophoretic mobility-shift assay using HeLa/vaccinia LEF-1 nuclear extract (lane 2) but not with HeLa/vaccinia HA (lane 1). The LEF-1/TCRα25 complex was sensitive to competition with the wild-type oligonucleotide but was resistant to competition with the mutant oligonucleotide (lanes 3–10), demonstrating sequence-specific binding of recombinant LEF-1.

Incubation of the TCRα25 fragment with nuclear extract from the T-cell line 1200M resulted in the formation of a complex that comigrated with a complex containing in vitro-translated LEF-1 (Fig. 6b, lanes 2 and 3). The apparent affinity of the 1200M nuclear protein for the LEF-1-binding site is similar to that of recombinant LEF-1, as determined by competition of binding with wild-type and mutant TCRα25 oligonucleotides (lanes 4–11). To confirm that the complex obtained with 1200M nuclear extract contains LEF-1 or a closely related protein, we incubated the nuclear extract with anti-LEF-1 serum prior to the addition of the labeled TCRα25 oligonucleotide (Fig. 6c). Increasing amounts of serum inhibited the formation of the protein/TCRα25 complex (lanes 4 and 5). No inhibition was observed with preimmune serum (lanes 2 and 3). To control for the specificity of the anti-LEF-1 serum, we examined the effect of anti-LEF-1 serum on the binding of Oct-1 to its OCTA recognition sequence. No interference with binding was observed (lanes 9 and 10). Taken together, these data indicate that both recombinant and endogenous LEF-1 bind specifically to a site in the TCRα enhancer.

Functional importance of LEF-1

The dependence of cell type-specific TCRα enhancer function on the LEF-1-binding site was examined by inserting two copies of the wild-type or a mutated TCRα98 enhancer fragment (see Fig. 5a) 5′ of a tkCAT reporter gene construct containing a 109-bp promoter fragment of the herpes simplex virus (HSV) thymidine kinase (tk) gene linked to the chloramphenicol acetyltransferase (CAT) gene (see Materials and methods). The tkCAT construct contained a triple poly(A^+) site upstream of the inserted enhancer fragments to reduce translatable readthrough from vector sequences (Maxwell et al. 1989). Wild-type and mutant TCRα98/tkCAT constructs were transfected together with a Rous sarcoma virus (RSV)–luciferase gene construct as a transfection control into various cell lines (Fig. 7). These experiments indicated that mutation of the LEF-1-binding site decreased enhancer function by a factor of 5 and 10 in the T-cell lines EL4 and BW5147, respectively, and by a factor of 4 in PD36 pre-B cells. In contrast, the mutations did not

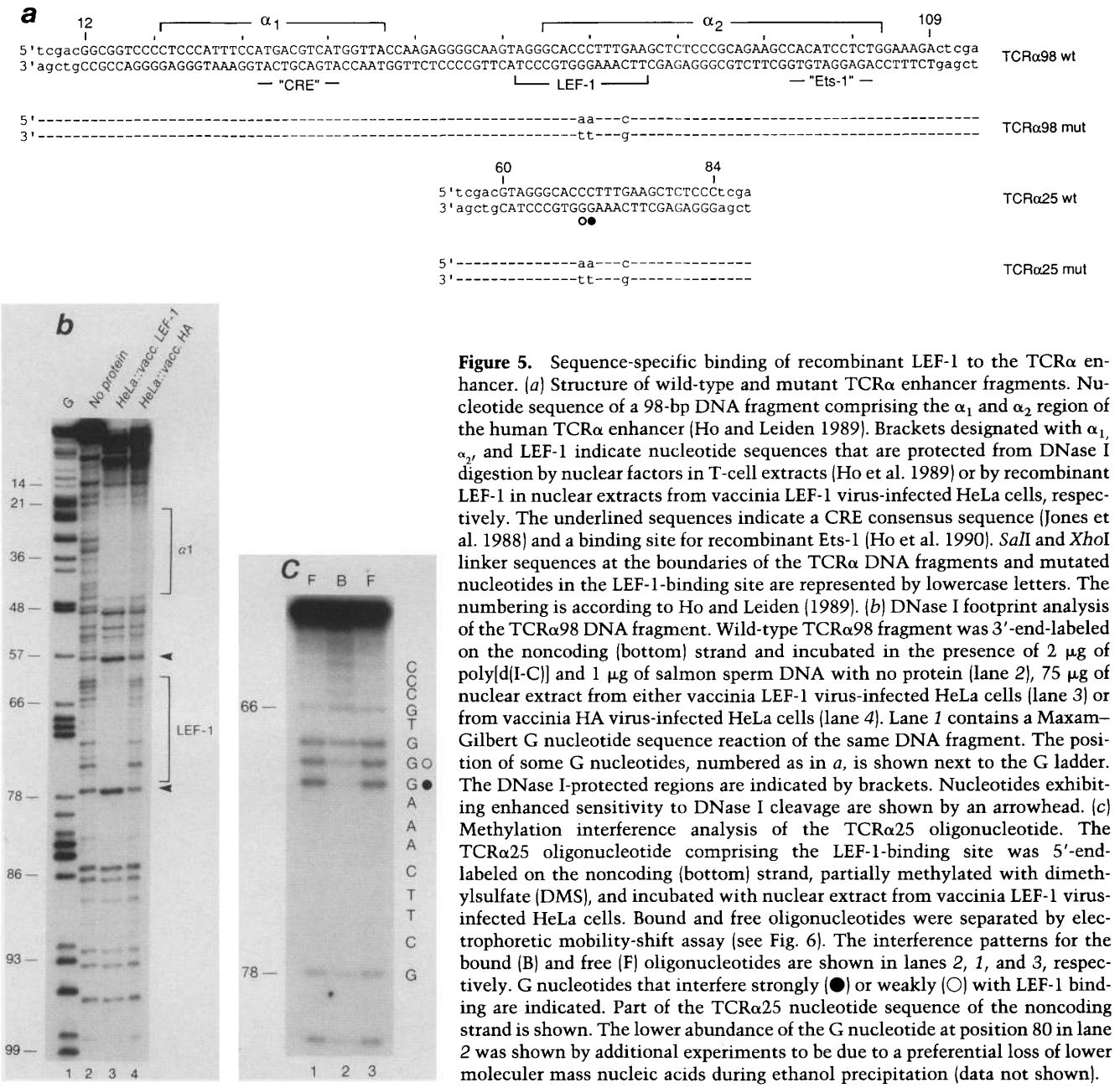

Figure 5. Sequence-specific binding of recombinant LEF-1 to the TCRα enhancer. (*a*) Structure of wild-type and mutant TCRα enhancer fragments. Nucleotide sequence of a 98-bp DNA fragment comprising the α_1 and α_2 region of the human TCRα enhancer (Ho and Leiden 1989). Brackets designated with α_1, α_2, and LEF-1 indicate nucleotide sequences that are protected from DNase I digestion by nuclear factors in T-cell extracts (Ho et al. 1989) or by recombinant LEF-1 in nuclear extracts from vaccinia LEF-1 virus-infected HeLa cells, respectively. The underlined sequences indicate a CRE consensus sequence (Jones et al. 1988) and a binding site for recombinant Ets-1 (Ho et al. 1990). *Sal*I and *Xho*I linker sequences at the boundaries of the TCRα DNA fragments and mutated nucleotides in the LEF-1-binding site are represented by lowercase letters. The numbering is according to Ho and Leiden (1989). (*b*) DNase I footprint analysis of the TCRα98 DNA fragment. Wild-type TCRα98 fragment was 3′-end-labeled on the noncoding (bottom) strand and incubated in the presence of 2 μg of poly[d(I-C)] and 1 μg of salmon sperm DNA with no protein (lane *2*), 75 μg of nuclear extract from either vaccinia LEF-1 virus-infected HeLa cells (lane *3*) or from vaccinia HA virus-infected HeLa cells (lane *4*). Lane *1* contains a Maxam–Gilbert G nucleotide sequence reaction of the same DNA fragment. The position of some G nucleotides, numbered as in *a*, is shown next to the G ladder. The DNase I-protected regions are indicated by brackets. Nucleotides exhibiting enhanced sensitivity to DNase I cleavage are shown by an arrowhead. (*c*) Methylation interference analysis of the TCRα25 oligonucleotide. The TCRα25 oligonucleotide comprising the LEF-1-binding site was 5′-end-labeled on the noncoding (bottom) strand, partially methylated with dimethylsulfate (DMS), and incubated with nuclear extract from vaccinia LEF-1 virus-infected HeLa cells. Bound and free oligonucleotides were separated by electrophoretic mobility-shift assay (see Fig. 6). The interference patterns for the bound (B) and free (F) oligonucleotides are shown in lanes *2*, *1*, and *3*, respectively. G nucleotides that interfere strongly (●) or weakly (○) with LEF-1 binding are indicated. Part of the TCRα25 nucleotide sequence of the noncoding strand is shown. The lower abundance of the G nucleotide at position 80 in lane *2* was shown by additional experiments to be due to a preferential loss of lower moleculer mass nucleic acids during ethanol precipitation (data not shown).

affect the low level of CAT expression obtained in M12 B cells, J558 plasmacytomas, and NIH-3T3 fibroblasts. Thus, the maximal TCRα98 enhancer activity depends on the LEF-1-binding site and is observed only in cells that contain LEF-1. The mutation of the LEF-1-binding site in the context of a single copy of the TCRα98 enhancer decreases enhancer function in BW5147 cells by a factor of eight as well, although the overall level of enhancer activity is fivefold lower than that of the duplicated enhancer (data not shown). Interestingly, and in agreement with data concerning the activity of a multimerized TCF-1α-binding site (Waterman and Jones 1990), neither a single nor multimerized TCRα25 oligonucleotide could augment basal transcription from a minimal *fos* promoter (Berkowitz et al. 1989), indicating that the function of the LEF-1-binding site is dependent on the sequence context (data not shown).

The regulatory role of LEF-1 for TCRα enhancer function was examined by cotransfection of M12 B cells, lacking endogenous LEF-1, with the TCRα98/tkCAT reporter gene together with a cytomegalovirus (CMV)/LEF-1 effector plasmid (see Materials and methods). Forced expression of LEF-1 in B cells increased the activity of the wild-type TCRα98 enhancer by a factor of 3.5 relative to the activity of the mutant TCRα98 enhancer carrying a nonfunctional LEF-1-binding site (Fig. 7b). Nei-

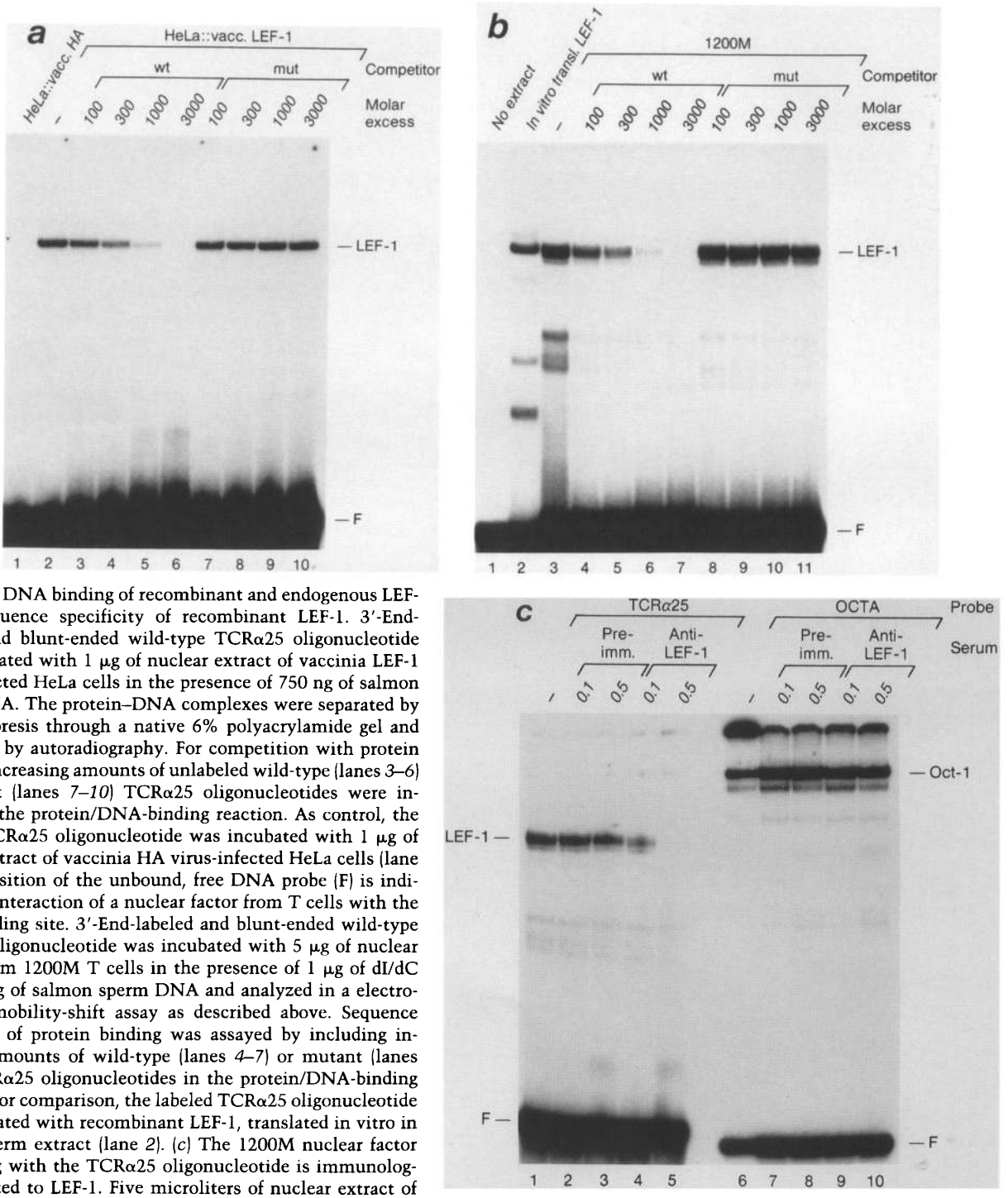

Figure 6. DNA binding of recombinant and endogenous LEF-1. (*a*) Sequence specificity of recombinant LEF-1. 3′-End-labeled and blunt-ended wild-type TCRα25 oligonucleotide was incubated with 1 μg of nuclear extract of vaccinia LEF-1 virus-infected HeLa cells in the presence of 750 ng of salmon sperm DNA. The protein–DNA complexes were separated by electrophoresis through a native 6% polyacrylamide gel and visualized by autoradiography. For competition with protein binding, increasing amounts of unlabeled wild-type (lanes *3–6*) or mutant (lanes *7–10*) TCRα25 oligonucleotides were included in the protein/DNA-binding reaction. As control, the labeled TCRα25 oligonucleotide was incubated with 1 μg of nuclear extract of vaccinia HA virus-infected HeLa cells (lane *1*). The position of the unbound, free DNA probe (F) is indicated. (*b*) Interaction of a nuclear factor from T cells with the LEF-1-binding site. 3′-End-labeled and blunt-ended wild-type TCRα25 oligonucleotide was incubated with 5 μg of nuclear extract from 1200M T cells in the presence of 1 μg of dI/dC and 500 ng of salmon sperm DNA and analyzed in a electrophoretic mobility-shift assay as described above. Sequence specificity of protein binding was assayed by including increasing amounts of wild-type (lanes *4–7*) or mutant (lanes *8–11*) TCRα25 oligonucleotides in the protein/DNA-binding reaction. For comparison, the labeled TCRα25 oligonucleotide was incubated with recombinant LEF-1, translated in vitro in a wheat germ extract (lane *2*). (*c*) The 1200M nuclear factor interacting with the TCRα25 oligonucleotide is immunologically related to LEF-1. Five microliters of nuclear extract of 1200M T cells was incubated with no serum (lane *1*), 0.1 and 0.5 μl of undiluted anti-LEF-1 serum (lanes *4* and *5*), or preimmune serum (lanes *2* and *3*) prior to addition of the 3′-end-labeled and blunt-ended wild-type TCRα25 oligonucleotide. The protein–DNA complexes and unbound, free DNA probe (F) were separated in an electrophoretic mobility-shift assay. One microgram of nuclear extract of 1200M T cells was incubated with no serum (lane *6*), preimmune serum (lanes *7* and *8*), or undiluted anti-LEF-1 serum (lanes *9* and *10*), prior to addition of a 5′-end-labeled wild-type OCTA oligonucleotide.

ther the activity of the wild-type nor that of the mutant TCRα98 enhancer was affected by the cotransfected control effector plasmid CMV/inv LEF-1 containing the LEF-1 cDNA insert in an inverse orientation. In contrast, transfection of the CMV/LEF-1 effector plasmid into NIH-3T3 fibroblastic cells did not increase the activity of a cotransfected TCRα98/tkCAT reporter gene (data not shown). Possibly, negative regulation in nonlym-

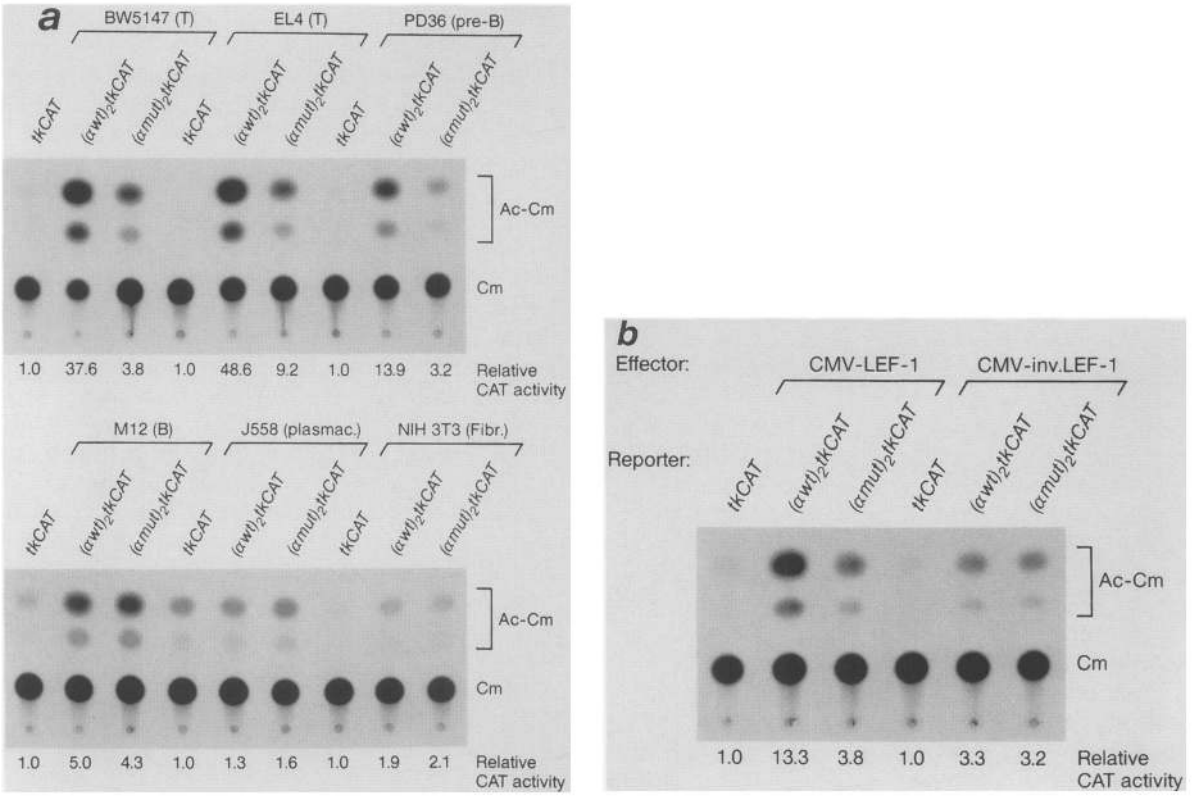

Figure 7. LEF-1 participates in regulating TCRα enhancer activity. (*a*) Dependence of the TCRα98 enhancer on the LEF-1-binding site parallels the expression pattern of LEF-1. ptkCAT and the derivative pTCRα98/tkCAT plasmids, carrying a duplicated wild-type (αwt) or mutant (αmut) TCRα98 enhancer fragment (see Fig. 5a) at position −109 of the HSV tk promoter, were transfected into various cell lines. pRSV–luciferase was included in all transfection experiments as an internal standard. A representative CAT assay, separating the acetyl–chloramphenicol (Ac-Cm) reaction products and chloramphenicol (Cm) by thin-layer chromatography, is shown, and the average relative CAT levels, which were determined by multiple transfections and normalized to the luciferase level, are indicated. The variability between independent experiments was <20%. (*b*) Expression of recombinant LEF-1 augments TCRα enhancer function in a LEF-1-binding site-dependent manner. M12 B-lineage cells, lacking endogenous LEF-1, were cotransfected with the ptkCAT or pTCRα98/tkCAT reporter plasmids together with a CMV/LEF-1 effector plasmid containing the GL1 cDNA linked to the CMV enhancer and promoter. The ratio of reporter–effector plasmid was 1 : 10. The control effector plasmid CMV/inv LEF-1 contains the GL1 cDNA linked to the CMV enhancer–promoter in an inverse orientation. CAT activity was assayed and normalized to the activity of a cotransfected RSV–luciferase gene.

phoid cells prevents LEF-1 from augmenting TCRα98 enhancer function. Alternatively, the function of LEF-1 may be dependent on a lymphoid-specific modification or cooperation with other lymphoid-specific factors. Whatever mechanisms restrict LEF-1 function to lymphoid cells, our experiments indicate that LEF-1 participates in the regulation of cell type-specific function of the TCRα enhancer.

Discussion

In this study we identified a murine regulatory gene that is expressed specifically in pre-B and T lymphocytes. This gene, termed LEF-1, encodes a sequence-specific DNA-binding protein that recognizes a functionally important site in the TCRα enhancer. A regulatory role of LEF-1 in TCRα-enhancer function is based on three arguments. First, transfection of a LEF-1 cDNA expression vector into a mature B-cell line increases the expression of a cotransfected reporter gene, containing a TCRα enhancer, in a LEF-1-binding site-dependent manner. Second, the LEF-1-binding site contributes to the function of this TCRα enhancer. Third, the cell type-specific pattern of maximal enhancer activity parallels the expression of LEF-1. The cell type specificity of both TCRα and TCRβ enhancers, however, is less confined than the expression pattern of their associated genes, suggesting that additional regulation is required to govern the ultimate developmental expression pattern of these genes. Recently, the TCRα gene was shown to contain silencers that appear to restrict gene expression to the $\alpha\beta^+$ subset of T lymphocytes (Winoto and Baltimore 1989b).

The expression of LEF-1 in pre-B and T cells raises the question as to whether LEF-1 is involved in the regulation of other genes that are expressed in early stage B cells and/or T-lineage cells. The binding site of LEF-1 in the human TCRα enhancer coincides with the binding

site of the recently identified and purified nuclear factor TCF-1α (Waterman and Jones 1990). TCF-1α was shown to bind to related sequences associated with the IgH enhancer and the promoters of the *lck*, *CD3γ*, and *CD3δ* genes and human immunodeficiency virus (HIV) (Waterman and Jones 1990). Therefore, we expect LEF-1 to bind to these sites as well. In addition to the coincidence of their recognition site in the TCRα enhancer, the relationship between LEF-1 and TCF-1α is extended further by the similarity of their apparent molecular masses, which are 54 kD for LEF-1 and 53–57 kD for TCF-1α (Waterman and Jones 1990). Moreover, TCF-1α was detected in T-cell lines but not in mature B-cell lines and nonlymphoid cells, which is consistent with the pre-B- and T-cell-specific expression pattern of LEF-1. Finally, a double mutation in the TCRα enhancer that abrogates binding of TCF-1α was recently shown to drastically decrease the function of a duplicated enhancer (Waterman and Jones 1990). Because different proteins can potentially recognize the same nucleotide sequence (Staudt et al. 1986; Scholer et al. 1989; Mitchell and Tjian 1989), further analysis of TCF-1α will be required to determine its relationship to LEF-1.

Although our data are compatible with a putative activator function of LEF-1, neither a single nor multimerized binding sites for LEF-1 augment basal transcription from a minimal *fos* promoter. Instead, function of the LEF-1-binding site can only be detected in the context of other factor-binding sites. This observation suggests either that LEF-1 is dependent on other DNA-binding proteins to bind with high affinity and to exert its function as a transcription factor or that LEF-1 stimulates transcription indirectly by affecting the binding or activity of other transcription factors. A comparison of the formation and stability of LEF-1/DNA complexes generated with nuclear extracts or in vitro-translated LEF-1 and various TCRα enhancer fragments does not indicate a dependence of LEF-1 binding on other factors or factor-binding sites (data not shown). Moreover, the nucleotide sequence context of the LEF-1-binding site is different in the TCRα and TCRβ enhancers. Therefore, we favor the view that LEF-1 may alter the DNA binding or action of other transcription factors at these enhancers.

In addition to its role in the activation of gene expression, LEF-1 may also be involved in negative regulation of gene expression. Recently, a multimerized TCRα enhancer fragment containing the α_2 footprint (see Fig. 5a), which includes the LEF-1-binding site and a binding site for Ets-1 (Ho et al. 1990), was shown to repress gene expression by antagonizing the function of a linked SV40 or RSV enhancer in a T-cell-specific manner (Ho and Leiden 1990b). The molecular components for this repression, however, have not yet been identified, and the putative involvement of LEF-1 has not been examined.

Although our experiments indicate an important role of LEF-1 for cell type-specific TCRα enhancer function, LEF-1 is unlikely to be the only cell type-specific regulatory protein involved. First, mutation of the LEF-1-binding site in the TCRα98 DNA fragment decreases enhancer function only to 10%. The mutant TCRα98 enhancer still displays some degree of cell type specificity because residual enhancer activity can be detected in lymphocytes (excluding plasmacytomas). This observed pattern of enhancer function in lymphocytes parallels the expression of *ets-1* (J. Hagman and R. Grosschedl, unpubl.), which has been shown previously to interact with a site 3′ of the LEF-1-binding site (Ho et al 1990). Second, multiple dispersed point mutations in the TCRα_2 footprint, which most likely abrogate binding of both LEF-1 and Ets-1, do not affect the function of a larger TCRα enhancer fragment comprising additional factor-binding sites (Ho and Leiden 1990a). Nevertheless, our data suggest that LEF-1 encodes a pre-B- and T-lymphocyte-specific DNA-binding protein that interacts with a specific site in the TCRα enhancer to participate in the developmental regulation of enhancer function.

The amino acid homology of LEF-1 with the nonhistone HMG-1 protein establishes LEF-1 as a new member of the family of HMG box proteins. In contrast to HMG-1, which is a nonspecific DNA-binding protein (for review, see van Holde 1989), LEF-1 appears to bind DNA in a sequence-specific manner. The relationship between LEF-1 and HMG-1 is reminiscent of that of the prokaryotic proteins IHF and HU which, despite their extensive amino acid homology, bind DNA specifically and nonspecifically, respectively (Yang and Nash 1989). In this respect, LEF-1 also differs from hUBF, another member of the family of HMG box proteins, whose sequence-specific DNA binding to the rRNA promoter was shown to be assisted by the interacting protein SL1 (Bell et al. 1990). We have no evidence that LEF-1 requires an interaction with another protein to bind DNA in a sequence-specific manner. Possibly, the HMG box of LEF-1 evolved into a DNA-binding domain that can recognize a specific nucleotide sequence independent of other proteins. Experiments examining the HMG box of LEF-1 as the putative DNA-binding domain indicated that 94 amino acids comprising the HMG homology are both necessary and sufficient for sequence-specific recognition of the LEF-1-binding site (A. Amsterdam and R. Grosschedl, in prep.).

In conclusion, we have identified a gene that encodes a developmentally regulated sequence-specific DNA-binding protein. In addition to its regulatory role for TCRα enhancer function, we anticipate LEF-1 to participate in the regulation of other genes that specify the lymphocyte phenotype. To unravel the function of LEF-1 for lymphocyte differentiation and mouse development, disruption of this gene in the mouse germ line (Mansour et al. 1988) or expression of dominant-negative mutants in transgenic mice (Herskowitz 1987) will ultimately be required.

Materials and methods

Isolation of cDNA clones

A set of lymphocyte-specific cDNA clones was isolated from a murine 70Z/3 pre-B-cell cDNA library in λgt11 (Ben-Neriah et al. 1986). Lymphocyte-specific clones were identified by screening duplicate bacteriophage lifts with [α-^{32}P]dCTP-labeled first-

strand cDNA generated from either 70Z/3 or MEL murine erythroleukemia poly(A)$^+$ RNA (Bergman et al. 1983; Ben-Neriah et al. 1986). Of 200,000 plaques that hybridized with the radiolabeled 70Z/3 cDNA but failed to hybridize with the radiolabeled MEL cDNA, 10,000 were picked as 16 pools of ~600. These pools were rescreened by the same method using radiolabeled cDNA derived from the murine T-cell lines BW5147 and EL4, the pre-B-cell 70Z/3, and MEL. Single plaques hybridizing with all three labeled lymphoid cDNAs but not with the labeled erythroid cDNA were identified, and the recombinant bacteriophages were isolated (Sambrook et al. 1989). cDNAs were subcloned into Bluescript (Stratagene) for sequencing of both strands of LEF-1 by the dideoxynucleotide method (Sambrook et al. 1989) using synthetic oligonucleotide primers and Sequenase (U.S. Biochemicals).

RNA analysis

Total RNA was prepared from C57BL6 mouse tissues, and total cytoplasmic RNA was prepared from cell lines as described (Bergman et al. 1983; Sambrook et al. 1989). Poly(A)$^+$ RNA was selected by passage over oligo(dT)-cellulose (BMB) (Sambrook et al. 1989). Poly(A)$^+$ RNA samples of 0.5 μg were separated on 1.0% agarose gels containing 2.2 M formaldehyde, transferred to Hybond-N nylon membranes (Amersham), UV-immobilized, and hybridized with a nick-translated 0.36-kb *Hin*cII DNA fragment from the GN8 cDNA (nucleotides 1158–1517 in Fig. 1b). Hybridization was at 42°C in 5× SSC, 20 mM $NaHPO_4$ at pH 6.7, 1× Denhardt's solution, 100 mg/ml of sheared/boiled salmon sperm DNA, and 50% formamide. Washing was in 0.5× SSC and 0.1% SDS at 65°C.

Antibody production, immunoblot analysis, and immunofluorescence

GN8 and GL1 polypeptides were synthesized in a rabbit reticulocyte lysate translation system (Promega) using RNA generated from Bluescript clones of GN8 and GL1 cDNAs with bacteriophage T7 RNA polymerase (BMB). Translation of the T7 transcripts from the GL1 cDNA was initiated at a synthetic in-frame AUG upstream of the cDNA insert, which resulted in an addition of 8 amino acids from the polylinker to the amino terminus of the GL1 polypeptide. Protein samples from cell lines were generated by lysing whole cells in SDS–sample buffer (Harlow and Lane 1988).

Rabbit antibodies were raised against the entire GL1-encoded polypeptide (amino acids 25–397), which was expressed in *Escherichia coli* using the pET vector system (Studier et al. 1990) and purified by preparative SDS–gel electrophoresis and electroelution (Harlow and Lane 1988). The serum was precleared of nonspecific antibodies by diluting it 1 : 10 with 3% BSA in PBS and incubating it with a protein blot of WEHI 231 B-cell extract for 18 hr at 4°C. SDS–gel electrophoresis and immunoblotting was as described (Harlow and Lane 1988), using the precleared antiserum at a final dilution of 1 : 3000. Detection of anti-LEF-1 antibodies was accomplished by incubation of the immunoblot with an alkaline phosphatase-conjugated second antibody (Promega).

For bright-field, rhodamine immunofluorescence, and DAPI fluorescence microscopy, 70Z/3 cells were allowed to settle onto poly-L-lysine-coated slides, fixed in 4% paraformaldehyde for 20 min, and permeabilized with 0.2% Triton X-100 for 4 min, and free formaldehyde groups were reduced in 50 mM NH_4Cl for 4 min (three times) and blocked in 0.5% BSA, 15% goat serum, and 0.05% Tween-20 for 5 min, all in PBS at room temperature. Incubation with the first antibody (anti-GL1 polypeptide) was for 40 min in a humidified chamber. DAPI staining of nuclei was at 0.1 μg/ml. A rhodamine-conjugated goat anti-rabbit antibody (Chemicon) was used for immunodetection.

Recombinant vaccinia viruses

GL1 cDNA encoding amino acids 25–397 of LEF-1 with an added in-frame ATG codon was inserted downstream of the H6 promoter in plasmid HES4, which contains a host-range gene of vaccinia virus (Perkus et al. 1989). Recombinant vaccinia/LEF-1 virus was selected by transfection of the recombinant HES4–GL1 gene construct into CV1 cells and coinfection with the deletion host range mutant vaccinia virus P293 (Perkus et al. 1989). Single plaques were isolated, and recombinant viruses were screened by immunoblot analysis of lysates of infected cells. Vaccinia/HA was generated in the same manner and was generously donated by Drs. George Kemble and Judith White.

Cell culture, nuclear extracts, and viral infections

Cells were grown to a density of 5×10^5 cells/ml. HeLa cells were grown in Joklik modified minimal essential medium (JMEM) supplemented with 10% equine serum. Lymphoid cell lines were grown in RPMI supplemented with 10% fetal calf serum and 50 μM 2-mercaptoethanol.

Nuclear extracts were prepared according to Dingham et al. (1983), with modifications described in Schreiber et al. (1989).

Infection of HeLa cells with recombinant vaccinia virus was performed 48 hr prior to the preparation of the nuclear extracts. For virus infection, 5×10^8 HeLa cells were pelleted and resuspended in 100 ml of JMEM with 2.5% equine serum, and virus was added at a m.o.i. of 1 pfu/cell.

Electrophoretic mobility-shift assays

DNA-binding reactions (10 μl) were carried out as described in the figure legends, containing 5 fmoles of end-labeled TCRα25 DNA probe (labeled on both 3′ ends by Klenow DNA polymerase fill-in reactions with [α-^{32}P]dCTP), poly[d(I-C)] and/or sonicated salmon sperm DNA, 10 mM HEPES (pH 7.9), 50 mM NaCl, 1 mM DTT, and 5% glycerol. Electrophoresis was performed through native 6% polyacrylamide gels in 25 mM Tris, 190 mM glycine, and 5 mM EDTA at 4°C.

DNase I footprint and methylation interference analysis

Nuclear extracts were incubated with 2 fmoles of single end-labeled DNA probe (isolated from a recombinant plasmid and labeled on one 3′ end by Klenow DNA polymerase fill-in reaction with [α-^{32}P]dCTP), for 30 min at room temperature in the presence of 2 μg of poly[d(I-C)], 1 μg of sonicated salmon sperm DNA, 10 mM HEPES (pH 7.9), 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM spermidine, and 10% glycerol. Samples were treated with DNase I at 50 μg/ml for 1 min, and the DNase I digestion was stopped by adjusting the reaction to 25 mM EDTA and 0.2% SDS. DNA samples were phenol-extracted, ethanol-precipitated, and separated by denaturing polyacrylamide gel electrophoresis .

For methylation interference analysis, the TCRα25 oligonucleotide representing the noncoding strand was 5′-end-labeled with [γ-^{32}P]ATP and polynucleotide kinase and annealed with a TCRα25-coding strand, and the double-stranded TCRα25 DNA probe was purified and partially methylated with dimethylsulfate (Maxam and Gilbert 1980). DNA-binding reactions (40 μl) were performed using 50 fmoles of single end-labeled TCRα25 probe, 24 μg of nuclear extract, 8 μg of poly[d(I-C)], 4 μg of sonicated salmon sperm DNA, 10 mM HEPES (pH 7.9), 50 mM

NaCl, 1 mM DTT, and 5% glycerol. Protein–DNA complexes were separated through 6% native polyacrylamide gels and processed as described (Singh et al. 1986).

DNA constructs, DNA transfections, and CAT assays

The tkCAT gene construct was generated by inserting a triple poly(A)$^+$ site fragment from SV40 (Maxwell et al. 1989) into the *Hin*dIII site of pmOTCO containing a 109-bp HSV tk promoter fragment linked to the CAT gene (DeFranco and Yamamoto 1986). To generate the TCRα98 DNA fragments, two 66-bp overlapping oligonucleotides, comprising part of the human TCRα enhancer (nucleotides 12–109; Ho and Leiden 1989) and containing a *Sal*I or *Xho*I linker sequence at their 5′ ends, were annealed, extended, and subcloned into a Bluescript vector. The nucleotide sequence of the cloned wild-type and mutant TCRα98 enhancer fragment (for nucleotide sequence, see Fig. 5a) was confirmed by sequencing, and the TCRα98 DNA fragments were isolated, dimerized, and inserted into the *Sal*I site of ptkCAT. The structure of the pRSV–luciferase gene construct is described in De Wet et al. (1987). For the construction of the CMV/LEF-1 and CMV/invLEF-1 effector plasmids, the isolated and blunt-ended GL1 cDNA insert (encoding amino acids 25–397) was inserted in the sense or antisense orientation into the *Sma*I site of pEV RF2 containing the CMV enhancer/promoter and the translation initiation region from the HSV tk gene (Matthias et al. 1989).

DNA transfections into cell lines were performed as described in Grosschedl and Baltimore (1985), using a DEAE–dextran/chloroquine procedure with 2 μg of DNA/ml (1.8 μg of reporter plus 0.2 μg of pRSV–luciferase, or 0.16 μg of reporter, 1.6 μg of effector, plus 0.2 μg of pRSV–luciferase). After 48 hr, cells were harvested to determine luciferase activity as described (Mangalam et al. 1989). CAT assays were performed as described (Gorman et al. 1982), using 3 μg of protein extract from M12, BW5417, and EL4 cells and 60 μg of protein extract from PD36, J558, and NIH-3T3 cells and incubating it in a CAT cocktail for 2 hr at 37°C. The exact amount of protein extract used was adjusted according to the luciferase activity of each sample. Acetylated [^{14}C]chloramphenicol was separated by thin-layer chromatography and autoradiographed. For quantitation of the acetylated [^{14}C]chloramphenicol, the chromatogram was exposed on a PhosphorImager (Molecular Dynamics), allowing direct determination of the radioactivity in each spot.

Acknowledgments

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Note added in proof

Since submission of the manuscript, the cloning of cDNAs encoding a human T-lymphocyte-specific HMG-box protein, termed TCF-1, was reported by Van de Wetering [*EMBO J.* **10:** 123–132 (1991)]. The central 78 amino acids of the HMG box of LEF-1 are 97% identical with those of TCF-1. However, the proteins appear to be encoded by distinct genes. In particular, the amino acid sequences of the proteins excluding the central region of the HMG box are different. Moreover, the nucleotide sequences of the human TCF-1 and murine LEF-1 in the actual region of the HMG box are divergent in the third nucleotide positions of most codons. Finally, the RNA blot analysis indicate a distinct pattern for TCF-1 and LEF-1 mRNAs. We also learned of the cloning of cDNAs encoding the human T-cell factor TCF-1α by Waterman et al. [*Genes & Dev.* **5:** 656–669], which appears to be the human homolog of LEF-1. The nucleotide sequence of murine LEF-1 has been submitted to the EMBL/GenBank data base libraries.

The originally submitted nucleotide sequence of LEF-1 contained an erroneous 13-necleotide insertion at position 2143 due to a subcloning artifact. The nucleotide sequence and the deduced amino acid sequence of LEF-1 shown in Figure 1 represent the corrected version.

References

Banerji, T., L. Olson, and W. Schaffner. 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell* **33:** 729–740.

Bell, S.P., H.-M. Jantzen, and R. Tjian. 1990. Assembly of alternative multiprotein complexes directs RNA promoter selectively. *Genes & Dev.* **4:** 943–954.

Ben-Neriah, Y., A. Bernards, M. Paskind, G.Q. Daley, and D. Baltimore. 1986. Alternative 5′ exons in c-*abl* mRNA. *Cell* **44:** 577–586.

Bergman, Y., S.J. Stewart, S. Levy, and R. Levy. 1983. Biosynthesis, glycosylation, and in vitro translation of the human T cell antigen lev-4. *J. Immunol.* **131:** 1876–1881.

Berkowitz, I.A., K.T. Riabowol, and M.Z. Gilman. 1989. Multiple sequence elements of a single functional class are required for cyclic AMP responsiveness of the mouse c-*fos* promoter. *Mol. Cell. Biol.* **9:** 4272–4281.

Blackman, M., J. Kappler, and P. Marrack. 1990. The role of the T cell receptor in positive and negative selection of developing T cells. *Science* **248:** 1335–1341.

Blackwell, T.K. and F.W. Alt. 1988. Immunoglobulin genes. In *Molecular immunology* (ed. B.D. Hames and D.M. Glover), pp. 1–60. IRL Press, Washington, D.C.

Cooper, M.D. and P.D. Burrows. 1989. B-cell differentiation. In *Immunoglobulin genes* (ed T. Honjo, F.W. Alt, and T.H. Rabbits), pp.1–22. Academic Press, London.

Davis, M.M. and P.J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature* **334:** 395–401.

DeFranco, D. and K.R. Yamamoto. 1986. Two different factors act separately or together to specify functionally distinct activities at a single transcriptional enhancer. *Mol. Cell. Biol.* **6:** 993–1001.

De Wet, J.R., K.V. Wood, M. DeLuca, D.R. Helinski, and S. Subramani. 1987. Firefly luciferase gene: Structure and expression in mammalian cells. *Mol. Cell. Biol.* **7:** 725–737.

Dingham, J.D., R.M. Lebovitz, and R.G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11:** 1475–1489.

Gerster, T., D. Picard, and W. Schaffer. 1986. During B-cell dif-

ferentiation enhancer activity and transcription rate of immunoglobulin heavy chain genes are high before mRNA accumulation. *Cell* **45:** 45–52.

Gillies, S.D., S.L. Morrison, V.T. Oi, and S. Tonegawa. 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell* **33:** 717–728.

Gorman, C.M., L.F. Moffat, and B.H. Howard. 1982. Recombinant genomes which express chloramphenicolacetyl transferase in mammalian cells. *Mol. Cell. Biol.* **2:** 1044–1051.

Gottschalk, L. and J.M. Leiden. 1990. Identification and functional characterization of the human T-cell receptor β gene transcriptional enhancer: Common nuclear proteins interact with the transcriptional regulatory elements of the T-cell receptor α and β genes. *Mol. Cell. Biol.* **10:** 5486–5495.

Grosschedl, R. and D. Baltimore. 1985. Cell-type specificity of immunoglobulin gene expression is regulated by at least three DNA sequence elements. *Cell* **41:** 885–897.

Gubbay, J., J. Collignon, P. Koopman, B. Capel, A. Economou, A. Münsterberg, N. Vivian, P. Goodfellow, and R. Lovell-Badge. 1990. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature* **346:** 245–250.

Harlow, E. and D. Lane. 1988. *Antibodies: A laboratory manual* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Henthorn, P., M. Kiledjian, and T. Kadesch. 1990. Two distinct transcription factors that bind the immunoglobulin enhancer μE5/κE2 motif. *Science* **247:** 467–470.

Herskowitz, I. 1987. Functional inactivation of genes by dominant negative mutations. *Nature* **329:** 219–222.

Ho, I.-C. and J.M. Leiden. 1990a. Regulation of the human T cell receptor α gene enhancer: Multiple ubiquitous and T cell-specific nuclear proteins interact with four hypomethylated enhancer elements. *Mol. Cell. Biol.* **10:** 4720–4727.

Ho, I.-C. and J.M. Leiden. 1990b. The Tα2 nuclear protein binding site from the human T cell receptor α enhancer functions as both a T cell-specific transcriptional activator and repressor. *J. Exp. Med.* **172:** 1443–1449.

Ho, I.-C., L.-H. Yang, G. Morle, and J.M. Leiden. 1989. A T-cell-specific transcriptional enhancer element 3′ of the Cα in the human T-cell receptor α locus. *Proc. Natl. Acad. Sci.* **86:** 6714–6718.

Ho, I.-C., N.K. Bhat, L.R. Gottschalk, T. Lindsten, C.B. Thompson, T.S. Papas, and J.M. Leiden. 1990. Sequence-specific binding of human Ets-1 to the T cell receptor α gene enhancer. *Science* **250:** 814–818.

Jantzen, H.-M., A. Admon, P.B. Bell, and R. Tjian. 1990. Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. *Nature* **344:** 830–836.

Jones, N.C., P.W.J. Rigby, and E.B. Ziff. 1988. *Trans*-acting protein factors and the regulation of eukaryotic transcription: Lessons from studies on DNA tumor viruses. *Genes & Dev.* **2:** 267–281.

Kelly, M., J. Burke, M. Smith, A. Klar, and D. Beach. 1988. Four mating-type genes control sexual differentiation in the fission yeast. *EMBO J.* **7:** 1537–1547.

Krimpenfort, P., R. de Jong, Y. Uematsu, Z. Dembic, S. Ryser, H. von Boehmer, M. Steinmetz, and A. Berns. 1988. Transcription of T cell receptor β-chain genes is controlled by a downstream regulatory element. *EMBO J.* **7:** 745–750.

Mangalam, H.J., V.R. Albert, H.A. Ingraham, M. Kapiloff, L. Wilson, C. Nelson, H. Elsholtz, and M.G. Rosenfeld. 1989. A pituitary POU domain protein, Pit-1, activates both growth hormone and prolactin promoters transcriptionally. *Genes & Dev.* **3:** 946–958.

Mansour, S.L., K.R. Thomas, and M.R. Capecchi. 1988. Disruption of the proto-oncogene *int-2* in mouse embryo-derived stem cells: A general strategy for targeting mutations to nonselectable genes. *Nature* **336:** 348–352.

Matthias, P., M.M. Muller, E. Schreiber, S. Rusconi, and W. Schaffner. 1989. Eukaryotic expression vectors for the analysis of mutant proteins. *Nucleic Acids Res.* **17:** 6418.

Maxam, A.M. and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65:** 499–550.

Maxwell, I.H., G.S. Harrison, W.M. Wood, and F. Maxwell. 1989. A DNA cassette containing a trimerized SV40 polyadenylation signal which efficiently blocks spurious plasmid-initiated transcription. *BioTechniques* **7:** 276–280.

McDougall, S., C.L. Peterson, and K. Calame. 1988. A transcriptional enhancer 3′ of the Cβ2 in the T cell receptor β locus. *Science* **241:** 205–208.

Mitchell, P.J. and R. Tjian. 1989. Transcriptional regulation in mammalian cells by sequence specific DNA binding proteins. *Science* **245:** 371–378.

Muller, M.M., S. Ruppsert, W. Schaffner, and P. Matthias. 1988. A cloned octamer transcription factor stimulates transcription from lymphoid-specific promoters in non-B cells. *Nature* **336:** 544–551.

Murre, C., P. Schonleber McCaw, and D. Baltimore. 1989. A new DNA-binding and dimerization motif in immunoglobulin enhancer binding, daughterless, myoD, and myc proteins. *Cell* **56:** 777–783.

Nelson, K., E. Mather, and R. Perry. 1984. Lipopolysaccharide-induced transcription of the κ immunoglobulin locus occurs on both alleles and is independent of methylation status. *Nucleic Acids Res.* **12:** 1911–1923.

Perkus, M.E., K. Limbach, and E. Paoletti. 1989. Cloning and expression of foreign genes in vaccinia virus, using a host range selection system. *J. Virol.* **63:** 3829–3836.

Roman, C., J.S. Platero, J. Shuman, and K. Calame. 1990. Ig/EBP-1: A ubiquitously expressed immunoglobulin enhancer binding protein that is similar to C/EBP and heterodimerizes with C/EBP. *Genes & Dev.* **4:** 1404–1415.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Scheidereit, C., J.A. Cromlish, T. Gerster, K. Kawakami, C. Balmaceda, R.A. Currie, and R.G. Roeder. 1988. A human lymphoid-specific transcription factor that activates immunoglobulin genes is a homeo-box protein. *Nature* **336:** 551–557.

Scholer, H.R., A.K. Hatzopoulos, R. Balling, N. Suzuki, and P. Gruss. 1989. A family of octamer-specific proteins present during mouse embryogenesis: Evidence for germline-specific expression of an Oct factor. *EMBO J.* **8:** 2543–2550.

Schreiber, E., P. Matthias, M.M. Muller, and W. Schaffner. 1989. Rapid detection of octamer binding proteins with "mini-extracts" prepared from a small number of cells. *Nucleic Acids Res.* **17:** 6419.

Sinclair, A.H., P. Berta, M.S. Palmer, J.R. Hawkins, B.L. Griffiths, M.J. Smith, J.W. Foster, A.M. Frischauf, R. Lovell-Badge and P.N. Goodfellow. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* **346:** 240–244.

Singh, H., J.H. LeBowitz, A.S. Baldwin, and P.A. Sharp. 1986. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. *Nature* **319:** 154–158.

Staben, C. and C. Yanofsky. 1990. *Neurospora crassa a* mating-type region. *Proc. Natl. Acad. Sci.* **87:** 4917–4921.

Staudt, L.M., H. Singh, R. Sen, T. Wirth, P.A. Sharp, and D.

Baltimore. 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. *Nature* **323:** 640–643.

Staudt, L.M., R.G. Clerc, J.H. LeBowitz, P.A. Sharp, and D. Baltimore. 1988. Cloning of a lymphoid-specific cDNA encoding a protein binding the regulatory octamer DNA motif. *Science* **241:** 577–580.

Studier, F.W., A.H. Rosenberg, J.J. Dunn, and J.W. Dubendorff. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185:** 60–89.

Takeda, J., A. Cheng, F. Mauxion, C.A. Nelson, R.D. Newberry, W.C. Sha, R. Sen, and D. Loh. 1990. Functional analysis of the murine T cell receptor β enhancer and characteristics of its DNA-binding proteins. *Mol. Cell. Biol.* **10:** 5027–5035.

van Holde, K.E. 1989. Chromatin (ed. A. Rich). Springer-Verlag Press, New York.

Waterman, M.L. and K.A. Jones. 1990. Purification of TCR-1α, a T-cell-specific transcription factor that activates the T-cell receptor Cα gene enhancer in a context-dependent manner. *New Biol.* **2:** 621–636.

Wen, L., J.-K. Huang, B.H. Johnson, and G.R. Reeck. 1989. A human placental cDNA clone that encodes non-histone chromosomal protein HMG-1. *Nucleic Acids Res.* **17:** 1197–1214.

Winoto, A. and D. Baltimore. 1989a. A novel, inducible and T cell-specific enhancer located at the 3′ end of the T cell receptor α locus. *EMBO J.* **8:** 729–733.

———. 1989b. αβ lineage-specific expression of the α T cell receptor gene by nearby silencers. *Cell* **59:** 649–655.

Yang, C.C. and H. Nash. 1989. The interaction of E. coli IHF protein with its specific binding sites. *Cell* **57:** 869–880.

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