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Indistinguishable Nuclear Factor Binding to Functional Core Sites of the T-Cell Receptor δ and Murine Leukemia Virus Enhancers

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We have previously shown that the δE3 site is an essential element for transcriptional activation by the human T-cell receptor (TCR) δ enhancer and identified two factors, NF-δE3A and NF-δE3C, that bound to overlapping core (TGTGGTTT) and E-box motifs within δE3. In this study, we show that protein binding to the core motif is necessary but not sufficient for transcriptional activation by the δE3 element. In contrast, protein binding to the E-box motif does not contribute significantly to enhancer activity. A similar core motif present within the enhancers of T-cell-tropic murine retroviruses has been shown to contribute to transcriptional activity of the viral long terminal repeat in T lymphocytes and to viral T-cell tropism. We therefore determined the relationship between the nuclear factors that bind to the TCR δ and Moloney murine leukemia virus core motifs. On the basis of electrophoretic mobility shift binding and competition studies, biochemical analysis of affinity-labeled DNA-binding proteins, and the binding of a purified core binding factor, the proteins that bound to the TCR δ core site were indistinguishable from those that bound to the murine leukemia virus core site. These data argue that DNA-binding proteins that interact with the core site of murine leukemia virus long terminal repeats and contribute to viral T-cell tropism also play an essential role in the T-cell-specific expression of cellular genes.

The rearrangement and expression of T-cell receptor (TCR) genes is regulated in a time- and lineage-specific fashion during T-lymphocyte development (5, 16). In order to elucidate the basis for developmental regulation, much effort has focused on identification of the *cis*-acting DNA sequence elements that are required to drive the transcription of these genes as well as the transcription factors that functionally interact with such sequences. These studies have resulted in the characterization of numerous T-cell-specific, lymphoid-specific, and ubiquitously expressed factors that activate transcription via their interaction with specific sites within enhancer elements that flank TCR constant-gene segments (reviewed in reference 12).

We have previously identified and characterized a T-cell-specific transcriptional enhancer located within the $J_\delta 3$-C_δ intron of the human TCR δ gene (17). We further defined a 30-bp element within the enhancer, denoted δE3, that binds multiple nuclear factors and is essential for significant transcriptional activation by the enhancer (18). Within δE3, overlapping binding sites were defined for two distinct factors, NF-δE3A and NF-δE3C. NF-δE3A is found in T-cell nuclear extracts and in some B-cell nuclear extracts and binds to a DNA sequence motif, TGTGGTTT, that is highly related to the viral core consensus sequence present in the enhancers of many animal viruses (20). NF-δE3C is ubiquitously expressed and binds to an overlapping DNA sequence element that is related to the immunoglobulin E-box motif (24). The binding site for a second ubiquitously expressed δE3 binding factor, NF-δE3B, is distinct from those of NF-δE3A and NF-δE3C but has not been precisely mapped. We showed that the introduction of a 3-bp substitution that inhibits the binding of NF-δE3A and NF-δE3C but does not affect binding of NF-δE3B eliminates transcriptional activation of the $V_\delta 1$ promoter by a single copy of the δE3 site and reduces transcriptional activation of this promoter by the intact TCR δ enhancer by 90 to 95% (18). From the restricted expression pattern of NF-δE3A, we suggested that this factor, rather than NF-δE3C, was essential for transcriptional activation through the δE3 element.

Studies of T-cell-tropic murine retroviruses have demonstrated that the viral core motif contributes to the transcriptional activity of the viral long terminal repeat (LTR) in T lymphocytes (2, 22, 26) and is a powerful determinant of the observed T-cell tropism of these viruses (21). Factors present in T-cell nuclear extracts (i.e., SL3-3 enhancer-binding factor-1 [SEF-1] and core binding factor [CBF]) that bind to the core motifs of a number of murine retroviruses have been described (2, 26–28). Recently, CBF has recently been purified to homogeneity from nuclear extracts prepared from calf thymus tissue (28). In addition, a factor called polyomavirus enhancer-binding protein 2 (PEBP2) that binds with similar specificity to the core site of the polyomavirus enhancer has recently been purified from nuclear extracts of H-*ras*-transformed NIH 3T3 fibroblasts (9).

In this report, we further examine transcriptional activation through the δE3 site of the TCR δ enhancer and explore the relationship between NF-δE3A and factors that bind to the core motif of murine leukemia virus enhancers. Our results indicate that the binding of NF-δE3A, rather than NF-δE3C, correlates with transcriptional activation through the δE3 site. An intact binding site for NF-δE3A is necessary for transcriptional activation through δE3 but is nevertheless not sufficient for transcriptional activation through this site. Furthermore, we show by multiple criteria that the factors that bind to the δE3 core element are indistinguishable from

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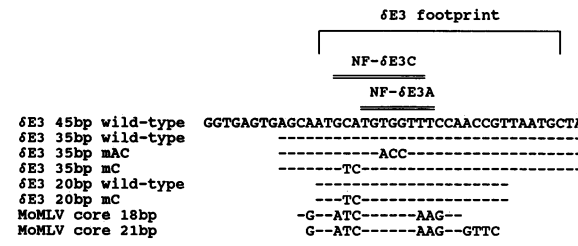

FIG. 1. Nuclear protein-binding sites tested. The actual oligonucleotides used in this study included flanking sequences appended to facilitate cloning and radiolabeling, as described in Materials and Methods. The extents of the δE3 binding site, as defined by DNase I footprinting, and the NF-δE3A and NF-δE3C binding sites, as defined by methylation interference, are shown. mAC designates a mutation that diminishes the formation of complexes δE3A and δE3C. mC designates a mutation that diminishes the formation of complex δE3C.

the factors that bind to the Moloney murine leukemia virus (MoMLV) core element. Together, these data argue that cellular genes specifically expressed in T lymphocytes and viral genes of T-cell-tropic retroviruses are regulated by common DNA-binding proteins.

MATERIALS AND METHODS

Plasmids and oligonucleotides. The binding sites tested in this study are shown in Fig. 1. Complementary oligonucleotides representing the 35-bp δE3 and $\delta E3^{mAC}$ sites (see Fig. 1 legend for nomenclature) were 40 nucleotides long, including appended *Bam*HI- and *Xba*I-compatible overhangs. Complementary oligonucleotides representing the 35-bp $\delta E3^{mC}$ site were 39 nucleotides long, including appended *Bam*HI-compatible overhangs. Complementary oligonucleotides representing the 20-bp δE3 and $\delta E3^{mC}$ sites were 24 nucleotides long, including *Xba*I-compatible overhangs. Complementary oligonucleotides representing the 18-bp MoMLV core site were 18 nucleotides long and generated blunt ends. The 45-bp δE3 site was generated by using the oligonucleotide 5′-ACTCTAGCATTAACGGTTGGAAACC ACATGCATTGCTCACTCACC-3′ and a 15-mer primer, 5′-GGTGAGTGAGCAATG-3′, for the synthesis of the second strand, as described previously (18). The 21-bp MoMLV core site was embedded within δE3 site-flanking sequence to generate the 45-mer 5′-actctagcaGAACTGCTTACCACAG ATATCcattgctcactcacc-3′ (uppercase letters represent MoMLV sequence, and lowercase letters represent δE3 site-flanking sequence); second-strand synthesis was accomplished by using the 15-mer primer noted above. The plasmid $V_8$1-CAT and versions of this plasmid carrying monomeric and trimeric 35-bp δE3 site, monomeric 35-bp $\delta E3^{mAC}$ site (previously called mδE3), and the intact 370-bp TCR δ enhancer (previously called fragment A) have been described before (18). Oligonucleotides representing the 35-bp $\delta E3^{mC}$ site were treated with T4 polynucleotide kinase (New England Biolabs, Beverly, Mass.), annealed, and ligated into *Bam*HI-digested and phosphatase-treated $V_8$1-CAT. Oligonucleotides representing the 20-bp δE3 site were treated with T4 polynucleotide kinase, annealed, and self-ligated, and trimers were gel purified and cloned into *Xba*I-digested and phosphatase-treated $V_8$1-CAT. The structures of all constructs were confirmed by dideoxynucleotide sequence analysis (19).

Transfections and CAT assays. For chloramphenicol acetyltransferase (CAT) assays, the human T-cell line Jurkat was transfected with CsCl-purified plasmid DNA by using Lipofectin (GIBCO-BRL, Gaithersburg, Md.) as described previously (17). Acetylation of [^{14}C]chloramphenicol (Du Pont-New England Nuclear, Boston, Mass.) was assayed as described before (17) and quantified with a Betascope (Betagen, Waltham, Mass.).

DNA-binding assays. The preparation of nuclear extracts, radiolabeling of binding-site probes with the Klenow fragment of DNA polymerase I (New England Biolabs) and [α-^{32}P]dGTP (Du Pont-NEN), and electrophoretic mobility shift assays (EMSAs) were done as described previously (18).

Analysis of affinity-labeled DNA-binding proteins. In situ UV-induced cross-linking with bromodeoxyuridine-substituted probes was performed as described previously (18). Affinity-labeled proteins were analyzed by partial proteolysis with *N*-chlorosuccinimide (Sigma Chemical Co., St. Louis, Mo.) as described before (13). Briefly, UV-cross-linked species were resolved by sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis (SDS–7.5% PAGE), and after autoradiography, appropriate slices of gel were excised. Gel slices were incubated with shaking at room temperature in 1 ml of each of the following solutions: water, twice for 10 min each; urea (1 g)-water (1 ml)-acetic acid (1 ml), twice for 10 min each; 15 mM *N*-chlorosuccinimide in urea-water-acetic acid, once for 30 min; water, twice for 10 min each; and Laemmli sample buffer (11), three times for 30 min each. Gel slices were then heated to 100°C and loaded onto a 10% polyacrylamide gel, and radiolabeled fragments were resolved by electrophoresis (11).

Partial purification of NF-δE3A. A DNA affinity column was prepared by coupling multimerized 20-bp δE3 site oligonucleotides to CNBr-activated Sepharose (Pharmacia, Piscataway, N.J.) as described before (8). Crude nuclear extract from the Jurkat T-cell line (3.5 mg of protein) was dialyzed against 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.4)–250 mM KCl–0.1% polyoxyethylene-9-lauryl ether (Sigma)–20% glycerol–0.2 mM EDTA–0.5 mM phenylmethylsulfonyl fluoride–0.5 mM dithiothreitol, incubated with 450 μg of poly(dI-dC) for 20 min at 4°C, and passed over a 0.5-ml affinity column. After extensive washing in the starting buffer, DNA-binding proteins were eluted first with 600 mM KCl and then with 1 M KCl in the starting buffer. NF-δE3C eluted primarily in the 600 mM fraction, whereas NF-δE3A eluted in the 1 M KCl fraction. The concentration of protein in the latter fraction was 30 μg/ml.

RESULTS

We previously used methylation interference to define overlapping binding sites for two distinct factors, NF-δE3A and NF-δE3C, within the δE3 element of the TCR δ enhancer (Fig. 1). A 3-bp substitution (changing TGG to ACC) was shown to abrogate the binding of NF-δE3A and diminish the binding of NF-δE3C and to dramatically reduce transcriptional activation by either a single copy of the δE3 element or an otherwise intact TCR δ enhancer (18). In order to discriminate between the roles of NF-δE3A and NF-δE3C binding in transcriptional activation, we generated oligonucleotides representing either 20 bp of wild-type δE3 sequence or a 20-bp site with a 2-bp substitution (CA to TC) predicted to selectively eliminate the binding of NF-δE3C ($\delta E3^{mC}$). We then used EMSA to test the ability of the $\delta E3^{mC}$ oligonucleotide to serve as a competitor for the

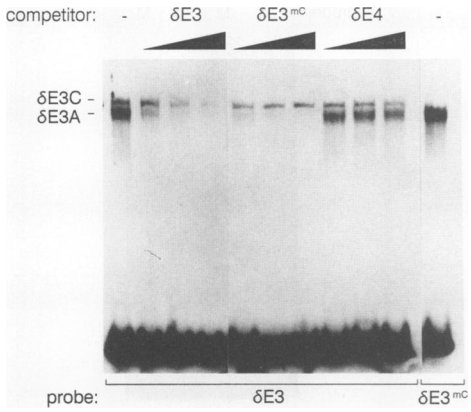

FIG. 2. Nuclear factor binding to a mutant δE3 site. Radiolabeled 20-bp δE3 and $\delta E3^{mC}$ binding sites (0.5 ng) were incubated with 4 μg of Jurkat nuclear extract in the absence of competitor or in the presence of increasing amounts of competitor (15-, 50-, and 150-fold molar excess). DNA-protein complexes were resolved by electrophoresis.

formation of complexes between the radiolabeled δE3 probe and factors in Jurkat T-cell nuclear extract (Fig. 2).

In the absence of any unlabeled oligonucleotide competitor, two complexes, δE3A and δE3C, were detected with the radiolabeled 20-bp δE3 site probe; this probe does not bind NF-δE3B. Both of the complexes detected were specific, since efficient competition was observed with an excess of unlabeled δE3 site but not with equivalent amounts of the heterologous δE4 site. The mutant $\delta E3^{mC}$ site was at least as efficient as the wild-type δE3 site in competing for the formation of the δE3A complex. However, the $\delta E3^{mC}$ site did not compete for the formation of the δE3C complex at any concentration tested. Furthermore, a radiolabeled $\delta E3^{mC}$ probe detected abundant levels of the δE3A complex but failed to detect the δE3C complex when used in a direct binding experiment (Fig. 2). Thus, the 2-bp substitution within the NF-δE3C binding site eliminates the binding of NF-δE3C but does not significantly affect the binding of NF-δE3A.

In order to evaluate the functional consequences of this mutation, wild-type and mutant sites were cloned upstream of the $V_\delta 1$ promoter in the enhancer-dependent test construct $V_\delta 1$-CAT, and constructs were transiently transfected into the Jurkat T-cell line to measure their activities (Fig. 3). Consistent with previous experiments, a trimer of a 35-bp δE3 site was a potent enhancer of transcription from the $V_\delta 1$ promoter (40.1-fold induction) and displayed a potency similar to that of the intact TCR δ enhancer (56.3-fold induction). A single copy of this site resulted in significant, although weaker, transcriptional activation as well (12.1-fold induction). In contrast, a single copy of a 35-bp δE3 site carrying the 3-bp substitution that affects both NF-δE3A and NF-δE3C binding ($\delta E3^{mAC}$) displayed no activity in this assay (0.3-fold induction). However, a single copy of a 35-bp mutant site that selectively eliminates NF-δE3C binding ($\delta E3^{mC}$) displayed enhancer activity that was essentially identical to that of the wild-type δE3 site (12.7-fold induction). Taken together, the results of EMSA and CAT assays with the $\delta E3^{mAC}$ and $\delta E3^{mC}$ sites argue pursuasively that binding of NF-δE3A rather than NF-δE3C is required for transcriptional activation through the δE3 site.

We also examined the activity of the 20-bp wild-type δE3 site in this experiment (Fig. 3). Strikingly, the 20-bp δE3 site failed to significantly augment transcription even when tested as a trimer (2.2-fold induction), despite the fact that this site efficiently bound NF-δE3A (Fig. 2). Since the 20-bp δE3 site is missing 6 bp at its 3′ end that are included in the δE3 footprint (Fig. 1), these data suggest that transcriptional activation through the δE3 site requires the cooperation of NF-δE3A with an additional factor that binds in the 3′ portion of the δE3 site. This activity might be mediated by the ubiquitous NF-δE3B, which does not bind to the 20-bp δE3 site in EMSA. Alternatively, this activity might be mediated by a distinct factor that has gone undetected in previous experiments.

NF-δE3A binds to a sequence within δE3, TGTGGTTT, that is highly related to the core consensus element present within the transcriptional enhancers of many animal viruses, including the long terminal repeats (LTRs) of the murine leukemia viruses. Previous studies have shown that the core sequence plays an important role in driving T-cell-specific gene expression and determining the T-cell tropism of the SL3-3 virus and MoMLV and have identified factors present in T-cell nuclear extracts (SEF-1 and CBF) that bind to the core element (2, 21, 22, 26–28). The core element present within the MoMLV LTR, TGTGGTAA, is similar to but nevertheless distinct from the related element within the TCR δ enhancer that is recognized by NF-δE3A. We therefore sought to examine the relationship between NF-δE3A and the factors that interact with the MoMLV core element.

We used EMSA to compare the nuclear complexes formed between factors present in crude Jurkat T-cell nuclear extract and radiolabeled δE3 and MoMLV core site oligonucleotide probes (Fig. 4). High levels of two specific complexes, δE3A and δE3C, were identified with a 45-bp δE3

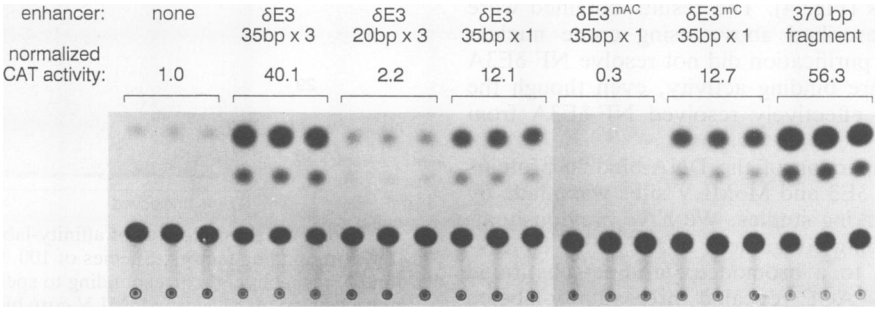

FIG. 3. Transcriptional activation by wild-type and mutant δE3 binding sites. Monomers and trimers of the indicated binding sites were tested for activation of the $V_\delta 1$ promoter in the test construct $V_\delta 1$-CAT. Constructs were transfected in triplicate into the Jurkat T-cell line, and values for percent chloramphenicol acetylation were averaged and then normalized to the activity of the enhancerless $V_\delta 1$-CAT construct.

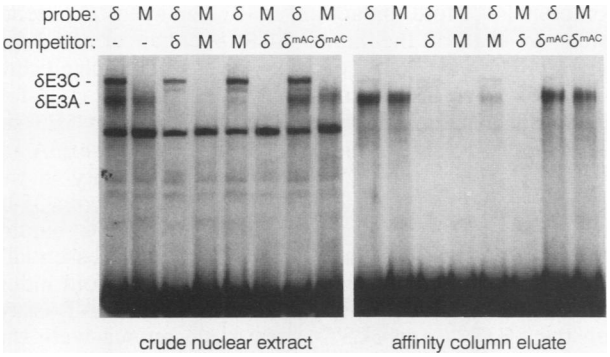

FIG. 4. Nuclear factor binding to δE3 and MoMLV core binding sites. Radiolabeled 45-mer δE3 (δ) and MoMLV core (M) binding sites (0.8 ng) were incubated with 2 μg of Jurkat nuclear extract or 60 ng of affinity column eluate in the absence of competitor or in the presence of a 50-fold molar excess of unlabeled δE3 20-bp (δ), MoMLV core 18-bp (M), or $\delta E3^{mAC}$ 35-bp (δ^{mAC}) competitors. DNA-protein complexes were resolved by electrophoresis. The δE3A and δE3C complexes are marked. The δE3B complex is normally detected with this δE3 probe and migrates slightly faster than the abundant nonspecific complex, but is not easily visualized in this experiment.

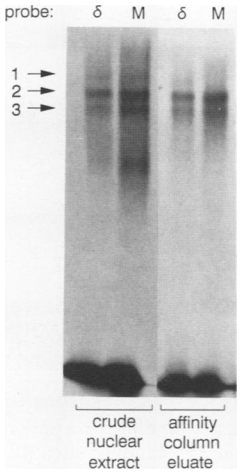

FIG. 5. Comparison of affinity-labeled NF-δE3A and MoMLV core binding factors. Bromodeoxyuridine-substituted, radiolabeled δE3 (δ) and MoMLV core (M) 45-mers were incubated with either Jurkat crude nuclear extract or affinity column eluate. Complexes were resolved by electrophoresis, UV irradiated in situ, and then analyzed by SDS–7.5% PAGE. Duplicate samples were processed for *N*-chlorosuccinimide cleavage (see Fig. 6). Affinity-labeled species labeled 1, 2, and 3 correspond to the similarly labeled 100-kDa, 90-kDa, and 78-kDa species, respectively, analyzed in Fig. 6.

site probe. The inclusion of an excess of unlabeled δE3 site oligonucleotide resulted in efficient competition for the δE3A complex and partial competition for the δE3C complex. Inclusion of an equivalent amount of unlabeled MoMLV core site oligonucleotide resulted in almost complete inhibition of the δE3A complex but failed to inhibit the δE3C complex. Unlabeled $\delta E3^{mAC}$ oligonucleotide did not compete in either case. Consistent with these observations, a radiolabeled MoMLV core site probe formed a DNA-protein complex with an electrophoretic mobility identical to that of δE3A, and the formation of this complex was inhibited by excess unlabeled δE3 and MoMLV core oligonucleotides but not by the $\delta E3^{mAC}$ oligonucleotide. This analysis suggests that NF-δE3A binds to both the δE3 site and the MoMLV core site.

A number of experiments were conducted to ascertain whether the complexes detected with the radiolabeled δE3 and MoMLV probes that were of identical electrophoretic mobility indeed contained the same DNA-binding proteins. A preparation of partially purified δE3 binding factors was generated by passing crude Jurkat nuclear extract over an affinity column consisting of multimerized δE3 site oligonucleotide and eluting with a step salt gradient. A fraction enriched for NF-δE3A was examined by EMSA with radiolabeled δE3 and MoMLV core site probes and the relevant unlabeled competitors (Fig. 4). The results obtained were identical to those described above using crude nuclear extract. Thus, partial purification did not resolve NF-δE3A from the MoMLV core binding activity, even though the step gradient elution effectively resolved NF-δE3A from NF-δE3C.

A more direct comparison of the DNA-binding proteins that interact with the δE3 and MoMLV sites was made by DNA-protein cross-linking studies. We have previously affinity labeled the binding component of NF-δE3A by UV-induced cross-linking to a bromodeoxyuridine-substituted δE3 probe (18). SDS-PAGE revealed three affinity-labeled polypeptides with mobilities in the 70- to 110-kDa range. To determine whether a similar array of polypeptides were bound by the MoMLV core site, we compared the polypeptides labeled by cross-linking to bromodeoxyuridine-substituted δE3 site and MoMLV core site probes by SDS-PAGE (Fig. 5). Highly similar arrays of polypeptides were detected by the two probes with either crude Jurkat nuclear extract or the partially purified fraction containing NF-δE3A activity (see above) used as the source of DNA-binding proteins.

To further explore the relationships among the various polypeptides that were affinity labeled by the two probes, the affinity-labeled polypeptides were individually excised from the polyacrylamide gel and partially proteolyzed by treatment with *N*-chlorosuccinimide, and the labeled cleavage products were reanalyzed by SDS-PAGE (Fig. 6). Analysis

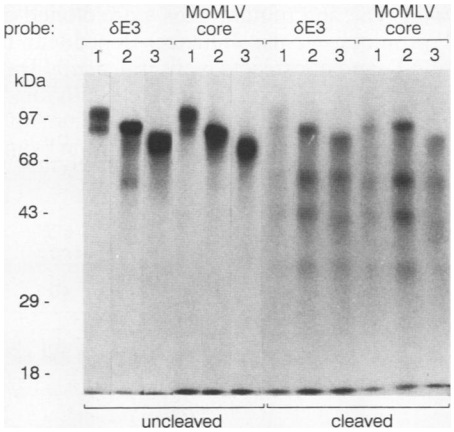

FIG. 6. Partial proteolysis of affinity-labeled NF-δE3A and MoMLV core binding factors. Species of 100, 90, and 78 kDa (lanes 1, 2, and 3, respectively, corresponding to species 1, 2, and 3 in Fig. 5) affinity labeled by δE3 and MoMLV core binding sites were excised from a 7.5% polyacrylamide gel and partially digested with *N*-chlorosuccinimide or mock digested, and the products were analyzed by SDS–10% PAGE.

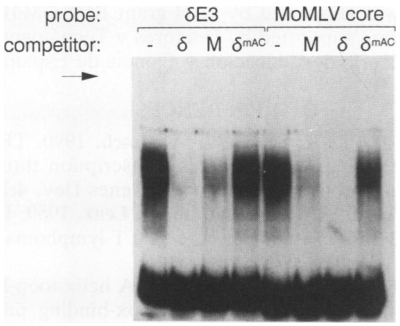

FIG. 7. Interaction of purified core binding factor with the δE3 and MoMLV core sites. Radiolabeled 45-mer δE3 and MoMLV core binding sites (1.0 ng) were incubated with 1 μl of purified CBF in the absence of competitor or in the presence of a 50-fold molar excess of unlabeled δE3 20-bp (δ), MoMLV core 18-bp (M), or $\delta E3^{mAC}$ 35-bp (δ^{mAC}) competitor. DNA-protein complexes were resolved by electrophoresis. The arrow marks the mobility of the δE3A complex. A specific complex of this mobility was detected at low levels in this experiment.

of the uncleaved material served to monitor the purity of the preparations of affinity-labeled polypeptides; only the largest δE3 labeled species showed significant contamination with other forms. Cleavage with *N*-chlorosuccinimide revealed a common pattern of affinity-labeled peptide fragments for all of the δE3-labeled and MoMLV core-labeled polypeptides. These data indicate that the three different size species affinity labeled by a single probe are all highly related to each other and that the proteins in Jurkat nuclear extract that bind to the MoMLV core site probe are likely identical to those that bind to the δE3 site probe.

A preparation of CBF that binds to the MoMLV core site has recently been purified from nuclear extracts of calf thymus (28). This preparation consists of a series of polypeptides in the 19- to 35-kDa range; heterogeneity may result from proteolytic degradation. We compared the ability of the purified CBF to bind to radiolabeled δE3 and MoMLV core site probes in an EMSA (Fig. 7). We found that purified CBF bound specifically to both probes, since competition was observed when an excess of the homologous binding site but not an excess of the $\delta E3^{mAC}$ site was used. Unlabeled δE3 served as a more efficient competitor for complex formation than unlabeled MoMLV core, suggesting that CBF binds with higher affinity to the former site. This binding hierarchy is identical to that observed for NF-δE3A in Jurkat nuclear extract. Notably, the mobility of the major complex formed between either probe and the purified calf thymus CBF was quite different from the mobility of the NF-δE3A complex. However, a complex with a mobility identical to that of NF-δE3A was detected at low levels in this experiment (arrow, Fig. 7).

DISCUSSION

In previous studies, we identified the δE3 site as an essential element for transcriptional activation by the human TCR δ enhancer and identified a DNA-binding factor, NF-δE3A, that is preferentially expressed in T lymphocytes and that specifically interacts with the motif TGTGGTTT within δE3 (17, 18). We also identified a ubiquitous factor, NF-δE3C, that binds to the overlapping motif GCATGTGGTT. In this study, we used mutagenesis to assess the relative contributions of NF-δE3A and NF-δE3C binding to transcriptional activation through the δE3 site. We found that transcriptional activation requires an intact binding site for NF-δE3A but not for NF-δE3C. Furthermore, we found that transcriptional activation requires additional sequences within δE3 that probably bind a distinct, uncharacterized nuclear factor. Thus, protein binding to the core site is necessary but not sufficient for transcriptional activation through δE3. We also explored the relationship between NF-δE3A and previously characterized nuclear factors that interact with the highly related core motif of T-cell-tropic murine retroviruses by comparing the factors that bound to the δE3 motif with those that bound to the MoMLV core site. On the basis of EMSA direct-binding and competition studies, biochemical analysis of affinity-labeled DNA-binding proteins, and the binding of a purified CBF, we could not distinguish the proteins that interact with the δE3 and MoMLV core sites. These data provide evidence that DNA-binding proteins that interact with the core site of murine leukemia virus LTRs and play an important role in viral T-cell tropism also play an essential role in the T-cell-specific expression of the TCR δ gene.

We and others have noted that the enhancers of numerous genes that are specifically expressed in T lymphocytes display sequences that are identical or very closely related to the δE3 site TGTGGTTT motif (10, 15, 18, 23, 27, 28). There are perfect matches to this sequence within the murine and human TCR β enhancers (6, 10, 15, 25), a sequence with a perfect match and one with a single mismatch (TGTGGTCT) within the murine TCR γ enhancer (23), and a sequence with a single mismatch (TGTGGTTA) within the CD3ε enhancer (4). Purified CBF binds to both sites within the TCR γ enhancer (28). Furthermore, the site within the CD3ε enhancer is identical to a binding site within the SL3-3 enhancer that binds a factor, called SEF-1, detected in T-cell nuclear extracts (26). This factor also interacts with the sequence TGTGGTTT (27) and is therefore probably identical to NF-δE3A. Thus, we predict that all of these sites are capable of interacting with the same DNA-binding proteins. These core sequences are located within segments of the TCR γ and TCR β enhancers that are important for transcriptional activity in T lymphocytes (6, 10, 15, 23, 25). Our previous results indicate that the introduction of a mutation into the core site of an otherwise intact TCR δ enhancer results in a 90 to 95% reduction in transcriptional activation by the enhancer (18). Thus, nuclear protein binding to core motifs may play a general and important role in T-cell-specific expression of cellular genes.

The present study argues that the ability of an isolated δE3 site to serve as an enhancer depends not only on protein binding to the core site, but probably also on protein binding to additional sequences within δE3 as well. One candidate for this protein is a previously detected δE3 binding factor, NF-δE3B, that displays a broad tissue distribution (18). Since NF-δE3B binding is not affected by mutations that diminish the binding of NF-δE3A, and since NF-δE3B does not bind to the 20-bp δE3 site, this factor likely binds to sequences within δE3 that are distinct from the binding site for NF-δE3A. It should be possible to address the role of NF-δE3B by defining its binding site in the methylation interference assay and testing mutations that interfere with binding for effects on transcriptional activation by the 35-bp δE3 site. As an alternative approach, random mutagenesis in the 3′ portion of δE3 has the potential to define the binding site for a factor that functionally cooperates with NF-δE3A but has not been detected in EMSA experiments.

Our data indicate that NF-δE3C binding to the E-box

motif that overlaps the NF-δE3A binding site does not play a significant role in transcriptional activation through δE3, since a mutation that selectively destroyed this site did not affect enhancer activity. We attempted to construct a reciprocal mutation that eliminated the binding of NF-δE3A without disturbing NF-δE3C binding but did not identify a mutation with these characteristics because of the extensive overlap of the two sites. As a result, it is formally possible that binding to the E motif could mediate weak transcriptional activation in this system. The ubiquitous factors USF, TFE3, and TFEB should all be capable of interacting with this site (1, 3, 7). USF is typically detected by EMSA with crude nuclear extracts, and it is therefore possible that NF-δE3C is identical to USF. Protein binding to the E motif might occur in vitro but not in vivo, a question that could be addressed by in vivo footprinting (14).

Our data indicate that both the δE3 and MoMLV core sites bind NF-δE3A from crude nuclear extracts of the Jurkat T-cell line and bind CBF purified from nuclear extracts of calf thymus. However, the precise relationship between NF-δE3A and CBF remains to be established. Affinity labeling of the DNA-binding component of NF-δE3A yields a series of protein-DNA complexes of 78, 90, and 100 kDa. While the precise contribution of the covalently bound oligonucleotide to the measured SDS-PAGE mobility of the complexes is not known, this contribution is likely to be no more than about 10 kDa. Thus, NF-δE3A is significantly larger than the predominant polypeptides in the CBF preparation, which range in size from 19 to 35 kDa. Extensive proteolysis of the polypeptides in the CBF preparation could be one explanation for this difference. In support of this interpretation, previous studies have shown that SEF-1, which is probably identical to NF-δE3A, is highly sensitive to proteolytic degradation and have shown that a number of proteases can convert SEF-1 to a fragment that forms a protein-DNA complex with a much higher electrophoretic mobility than the intact SEF-1-DNA complex (26). Since incubation of CBF with radiolabeled δE3 and MoMLV core site probes revealed low levels of a DNA-protein complex with a mobility identical to that of NF-δE3A (arrow, Fig. 7), it is possible that the CBF preparation contained small amounts of intact protein. We did not attempt to use partial proteolysis for a biochemical comparison of the predominant affinity-labeled NF-δE3A and CBF forms because the smallest fragment of affinity-labeled NF-δE3A generated by *N*-chlorosuccinimide treatment was 34 kDa, the same apparent size as the largest of the polypeptides in the CBF preparation.

NF-δE3A and CBF have similar relative affinities for the δE3 and MoMLV core sites, as both appear to bind the δE3 site TGTGGTTT more efficiently than the MoMLV site TGTGGTAA. Consistent with this result, 95% of the sites bound by CBF in an in vitro binding-site selection assay displayed a T in the seventh position (TGTGGTT) (3a). Thus, NF-δE3A and CBF display closely related DNA-binding specificities. However, a more definitive evaluation of the relationship between NF-δE3A and CBF will require either the purification and biochemical characterization of NF-δE3A or the use of antibodies raised against purified CBF to establish the immunological cross-reactivity of the two species.

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REFERENCES

1. **Beckman, H., L.-K. Su, and T. Kadesch.** 1990. TFE3: a helix-loop-helix protein that activates transcription through the immunoglobulin enhancer μE3 motif. Genes Dev. **4**:167–179.
2. **Boral, A. L., S. A. Okenquist, and J. Lenz.** 1989. Identification of the SL3-3 virus enhancer core as a T-lymphoma cell-specific element. J. Virol. **63**:76–84.
3. **Carr, C. S., and P. A. Sharp.** 1990. A helix-loop-helix protein related to the immunoglobulin E box-binding proteins. Mol. Cell. Biol. **10**:4384–4388.

3a. **Cherepennikova, I., S. Wang, and N. A. Speck.** Unpublished data.

4. **Clevers, H., M. Lonberg, S. Dunlap, E. Lacy, and C. Terhorst.** 1989. An enhancer located in a CpG-island 3′ to the TCR/CD3-ε gene confers T lymphocyte-specificity to its promoter. EMBO J. **8**:2527–2535.
5. **Davis, M. M., and P. J. Bjorkman.** 1988. T-cell antigen receptor genes and T-cell recognition. Nature (London) **334**:395–402.
6. **Gottschalk, L. R., 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.
7. **Gregor, P. D., M. Sawadogo, and R. G. Roeder.** 1990. The adenovirus major late transcription factor USF is a member of the helix-loop-helix group of regulatory proteins and binds to DNA as a dimer. Genes Dev. **4**:1730–1740.
8. **Kadonaga, J. T., and R. Tjian.** 1986. Affinity purification of sequence-specific DNA binding proteins. Proc. Natl. Acad. Sci. USA **83**:5889–5893.
9. **Kamachi, Y., E. Ogawa, M. Asano, S. Ishida, Y. Murakami, M. Satake, Y. Ito, and K. Shigesada.** 1990. Purification of a mouse nuclear factor that binds to both the A and B cores of the polyomavirus enhancer. J. Virol. **64**:4808–4819.
10. **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.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Leiden, J. M.** 1992. Transcriptional regulation during T-cell development: the α TCR gene as a molecular model. Immunol. Today **13**:22–30.
13. **Lischwe, M. A., and D. Ochs.** 1982. A new method for partial peptide mapping using *N*-chlorosuccinimide-urea and peptide silver staining in sodium dodecyl sulfate-polyacrylamide gels. Anal. Biochem. **127**:453–457.
14. **Mueller, P. R., and B. Wold.** 1989. In vivo footprinting of a muscle-specific enhancer by ligation-mediated PCR. Science **246**:780–786.
15. **Prosser, H. M., R. A. Lake, D. Wotton, and M. J. Owen.** 1991. Identification and functional analysis of the transcriptional enhancer of the human T cell receptor β gene. Eur. J. Immunol. **21**:161–166.
16. **Raulet, D. H.** 1989. The structure, function, and molecular genetics of the γ/δ T cell receptor. Annu. Rev. Immunol. **7**:175.
17. **Redondo, J. M., S. Hata, C. Brocklehurst, and M. S. Krangel.** 1990. A T cell-specific transcriptional enhancer within the human T cell receptor δ locus. Science **247**:1225–1229.
18. **Redondo, J. M., J. L. Pfohl, and M. S. Krangel.** 1991. Identification of an essential site for transcriptional activation within the human T-cell receptor δ enhancer. Mol. Cell. Biol. **11**:5671–5680.
19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463.
20. **Sassone-Corsi, P., and E. Borrelli.** 1986. Transcriptional regulation by trans-acting factors. Trends Genet. **2**:215–219.

21. **Speck, N. A., B. Renjifo, E. Golemis, T. N. Fredrickson, J. W. Hartley, and N. Hopkins.** 1990. Mutation of the core or adjacent LVb elements of the Moloney murine leukemia virus enhancer alters disease specificity. Genes Dev. **4**:233–242.
22. **Speck, N. A., B. Renjifo, and N. Hopkins.** 1990. Point mutations in the Moloney murine leukemia virus enhancer identify a lymphoid-specific viral core motif and 1,3-phorbol myristate acetate-inducible element. J. Virol. **64**:543–550.
23. **Spencer, D. M., Y.-H. Hsiang, J. P. Goldman, and D. P. Raulet.** 1991. Identification of a T-cell-specific transcriptional enhancer located 3′ of $C_\gamma 1$ in the murine T-cell receptor γ locus. Proc. Natl. Acad. Sci. USA **88**:800–804.
24. **Staudt, L. M., and M. J. Lenardo.** 1991. Immunoglobulin gene transcription. Annu. Rev. Immunol. **9**:373–398.
25. **Takeda, J., A. Cheng, F. Mauxion, C. A. Nelson, R. D. Newberry, W. C. Sha, R. Sen, and D. Y. 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.
26. **Thornell, A., B. Hallberg, and T. Grundstrom.** 1988. Differential protein binding in lymphocytes to a sequence in the enhancer of the mouse retrovirus SL3-3. Mol. Cell. Biol. **8**:1625–1637.
27. **Thornell, A., B. Hallberg, and T. Grundstrom.** 1991. Binding of SL3-3 enhancer factor 1 transcriptional activators to viral and chromosomal enhancer sequences. J. Virol. **65**:42–50.
28. **Wang, S., and N. A. Speck.** 1992. Purification of core-binding factor, a protein that binds the conserved core site in murine leukemia virus enhancers. Mol. Cell. Biol. **12**:89–102.