Regulation of T Cell Receptor \(\delta\) Gene Rearrangement by CBF/PEBP2

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Summary

We have analyzed transgenic mice carrying versions of a human T cell receptor (TCR)-\(\delta\) gene minilocus to study the developmental control of VDJ (variable/diversity/joining) recombination. Previous data indicated that a 1.4-kb DNA fragment carrying the TCR-\(\delta\) enhancer (E\(_\delta\)) efficiently activates minilocus VDJ recombination in vivo. We tested whether the transcription factor CBF/PEBP2 plays an important role in the ability of E\(_\delta\) to activate VDJ recombination by analyzing VDJ recombination in mice carrying a minilocus in which the \(\delta\)E3 element of E\(_\delta\) includes a mutated CBF/PEBP2 binding site. The enhancer-dependent VD to J step of minilocus rearrangement was dramatically inhibited in three of four transgenic lines, arguing that the binding of CBF/PEBP2 plays a role in modulating local accessibility to the VDJ recombinase in vivo. Because mutation of the \(\delta\)E3 binding site for the transcription factor c-Myb had previously established a similar role for c-Myb, and because a 60-bp fragment of E\(_\delta\) carrying \(\delta\)E3 and \(\delta\)E4 binding sites for CBF/PEBP2, c-Myb, and GATA-3 displays significant enhancer activity in transient transfection experiments, we tested whether this fragment of E\(_\delta\) is sufficient to activate VDJ recombination in vivo. This fragment failed to efficiently activate the enhancer-dependent VD to J step of minilocus rearrangement in all three transgenic lines examined, indicating that the binding of CBF/PEBP2 and c-Myb to their cognate sites within E\(_\delta\), although necessary, is not sufficient for the activation of VDJ recombination by E\(_\delta\). These results imply that CBF/PEBP2 and c-Myb collaborate with additional factors that bind elsewhere within E\(_\delta\) to modulate local accessibility to the VDJ recombinase in vivo.

The process of VDJ recombination assembles variable (V\(^1\)), diversity (D), and joining (J) gene segments at TCR and Ig loci during lymphocyte development, generating the diverse antigen receptor repertoires that characterize mature T and B lymphocytes (1–5). VDJ recombination is under stringent developmental control, as it is activated at individual antigen receptor loci with unique cell lineage-specific and developmental stage-specific properties. Thus, fully rearranged TCR genes are only generated in developing T lymphocytes, and fully rearranged Ig genes are only generated in developing B lymphocytes. Further, TCR-\(\beta\), -\(\gamma\), and -\(\delta\) rearrangement occurs earlier during thymocyte development than TCR-\(\alpha\) rearrangement (6), and IGH rearrangement occurs earlier in B cell development than Igk and \(\lambda\) rearrangement (1). Because all of these loci are thought to share both recombination signal sequences and the known components of the recombinase machinery, it is generally believed that these factors cannot account for locus-specific regulation of VDJ recombination. Rather, it is thought that locus-specific regulation is accomplished by modulating the accessibility of chromosomal recombination substrates to the recombinase (1–5).

The expression of fully rearranged TCR and Ig genes is controlled by a promoter flanking the V gene segment, and one or more transcriptional enhancers located adjacent to the C gene segment (7, 8). Recent studies have indicated that these cis-acting elements are also required for the developmental activation of VDJ recombination at individual antigen receptor loci. This has been accomplished by eliminating, mutating, or substituting enhancer or promoter elements within chromosomally integrated VDJ recombination substrates in transgenic mice (9–16), as well as by eliminating enhancer elements from endogenous antigen receptor loci by homologous recombination (17–23). These studies show that the efficiency of VDJ recombination is dramatically inhibited in the absence of a functional enhancer, and that the developmental activation of VDJ recombination can be modified by substitution of one enhancer for another.

Abbreviations used in this paper: D, diversity; J, joining; V, variable.
We have previously studied the developmental control of VDJ recombination in transgenic mice carrying a human TCR-δ gene minilocus (13, 14). Efficient VDJ recombination of this minilocus requires the presence of Eδ within the Jα-3-Cδ intron. Interestingly, the first step of minilocus rearrangement, V to D, occurs even in the absence of Eδ, whereas the second step of minilocus rearrangement, V to J, is Eδ dependent (13). Thus, a functional enhancer is critical for establishing J segment accessibility to the VDJ recombinase machinery. Furthermore, substitution of Eγ for Eδ within the minilocus reveals that Eγ and Eδ regulate both the T cell subset and developmental stage specificity of the VD to J step of minilocus rearrangement in a manner that mimics the developmental activation of Vδ1-DJδ and Vδ2 rearrangement, respectively, at the endogenous TCR-δ/δ locus (14). These results lead to the conclusion that Eδ and Eγ are indeed responsible for the developmental regulation of VDJ recombination at the endogenous TCR-δ locus.

The developmental properties of Eδ and Eγ presumably result from the binding of specific trans-acting factors to discrete sites within the enhancers. Therefore, to better understand the mechanism by which these enhancers control VDJ recombination, we have begun to introduce mutations into previously defined ds-acting enhancer elements within the context of the TCR-δ gene minilocus, and to measure the effects of these mutations on the process of VDJ recombination in vivo. Eδ was initially defined and functionally dissected in transient transfection and in vitro protein binding studies (24-28). These experiments identified an essential element of the enhancer, δE3, that contains adjacent binding sites for the transcription factors CBF/PEBP2 (29-31) and c-Myb (32). Intact binding sites for both CBF/PEBP2 (the “core” site) (25) and c-Myb (27) are required for transcriptional activation by Eδ. Because CBF/PEBP2 has been implicated in TCR-α, -δ, and -γ enhancer activity as well (30, 33-36), it appears to be a crucial and broadly important regulator of T cell development and T cell-specific gene expression. Mice carrying a homozygous mutation within the gene encoding one particular CBF/PEBP2 isoform (κB) display a very early defect in hematopoiesis and early embryonic lethality (37). Although these results clearly demonstrate an important role for CBF/PEBP2 in the development of early hematopoietic precursor cells, they do not provide information regarding subsequent molecular events that might be regulated by CBF/PEBP2 within developing thymocytes in vivo.

The present study was initiated to determine whether CBF/PEBP2 plays an important role in the developmental activation of TCR genes in vivo, by specifically testing its role in the activation of VDJ recombination by Eδ. We found that disruption of the δE3 core site significantly impairs the ability of Eδ to activate VDJ recombination within the TCR-δ gene minilocus, suggesting that CBF/PEBP2 is an important regulator of VDJ recombination in vivo. Since previous data had indicated an important role for c-Myb as well, we then asked whether a small fragment of Eγ carrying binding sites for these factors as well as GATA-3 was sufficient to activate VDJ recombination. We found that this was not the case, arguing that additional ds-acting elements of Eγ are also required to establish local accessibility to the VDJ recombinase.

Materials and Methods

Production of transgenic mice. The CBF/PEBP2 binding site mutation was generated by PCR using as a template the 1.4-kb wild-type Eγ subcloned into the XbaI site of pBluescript KS− (1.4EδBS). PCR overlap extension was performed as described (38) using mutagenic oligonucleotides ACOREM (AGGAGTGTGTGACCTTTCCAACCAG) and BCOREM (GGTTGGAAAGGTGATGATGTTTAC) along with EDR A (CTTTTAAAATTCTTCTAGCAAGC) and the reverse primer as outside primers. The final PCR product was digested with NsiI and BamH I to generate a 170-bp fragment of Eγ carrying the 3-bp change in δE3 that eliminates CBF/PEBP2 binding. The plasmid 1.4EδBS was also digested with PmI and NsiI to obtain an adjacent 590-bp fragment of Eγ. The two fragments were ligated together into PmI and BamH I cut 1.4EδBS. The structure of the resulting plasmid was determined by restriction mapping and dyeoxynucleotide sequence analysis. The 1.4-kb EγmCore was excised from this plasmid with XbaI and cloned into XbaI-digested, phosphatase-treated pBluescript carrying the previously described enhancerless minilocus (13).

A minilocus construct containing the δE3,4 region was generated as follows. A 60-bp NsiI-AluI fragment of Eγ (δE3,4) had been previously subcloned into PmI and EcoR V cut pBluescript KS−. The insert was excised from this plasmid by digestion with BamH I and Hind III and the ends were blunted by treatment with the Klenow fragment of E. coli DNA polymerase I. This fragment was then ligated into XbaI-digested, blunted, and phosphatase-treated pBluescript carrying the enhancerless minilocus. The structures of both minilocus constructs were confirmed by dyeoxynucleotide sequence analysis.

Minilocus DNA was purified as described previously (13), and was microinjected into fertilized C57BL/6 × SJL F2 eggs by the Duke University Comprehensive Cancer Center Transgenic Mouse Shared Resource. Progeny tail DNA samples were analyzed on Southern blots as described previously (13). Transgenes were maintained on a mixed C57BL/6 × SJL background. Copy number was determined by analysis of tail DNA on a slot blot (Schleicher and Schuell, Keene, N H) using a radiolabeled Cγc CDNA probe. Hybridization signals were quantitated relative to previously identified single copy integrants using a Betascope (Betagen, Wal-tham, MA).

Preparation and analysis of genomic DNA. Genomic DNA preparation, PCR, gel electrophoresis, blotting onto nylon membranes (Hybond-N; Amersham, Arlington Heights, IL or MAGNA nylon; Micron Separations Inc., Westboro, MA), and hybridization with 32P-labeled probes were performed as described previously (13). The amount of DNA template used in PCR reactions (2-12 ng) was adjusted based on the results of amplification using Cγc primers to account for differences in transgene copy number and PCR efficiency between samples, so that all PCR signals were maintained in the linear range. TCR-δ minilocus PCR primers 1 (VD1), 2 (VD2), 3 (JD1), 4 (JD3), 5 (CDA), and 6 (CDB), as well as the V3, V4, V5, and Cγc fragments used as probes to develop Southern blots of PCR products or genomic restriction digests, were described previously (13). These primers and probes allow detection of human TCR-δ minilocus sequences, but do not allow detection of endogenous murine TCR-δ locus sequences. Quantification of PCR signals was accomplished using either a

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Results

An Intact CBF/PEBP2 Binding Site Is Necessary for Efficient TCR-δ Gene Rearrangement In Vivo. The previously studied human TCR-δ gene minilocus is a 22.5-kb construct containing germline Vδ1, Vδ2, Dδ3, Jδ1, Jδ3, and Cδ gene segments, along with a wild-type version of Eδ within the Jδ3-Cδ intron (13) (Fig. 1A). The Vδ1 and Vδ2 coding segments carry mutations to prevent a rearranged transgene from encoding a TCR protein that might interfere with normal murine T cell development. Thus, the minilocus serves as a phenotypically neutral in vivo reporter of VDJ recombination.

For this study, we initially constructed a new version of the minilocus, referred to as EδmCore, that carries a three-basepair change in the δE3 core sequence within Eδ (Fig. 1A). The identical mutation was previously shown to eliminate CBF/PEBP2 binding to δE3 in vitro, and to eliminate transcriptional activation by Eδ in transient transfection experiments (25). Three transgenic founders, designated U, Y, and Z, were initially obtained. Breeding experiments indicated that in each of founders U and Z, the EδmCore minilocus was integrated at a single site in the mouse genome, whereas in founder Y there were two independently segregating transgene integration sites. As a result, four different EδmCore transgenic lines (U, Z, Y1, Y2) were established. As assessed by slot blot, the transgene copy numbers were: U, 8 copies; Z, 3 copies; Y1, 1 copy; and Y2, 2 copies. The single copy integrant in line Y1 was truncated so as to delete ~4–5 kb at the 3′ end of the minilocus. This truncation leaves Vδ1, Dδ3, Jδ3 gene segments, Eδ, and the first Cδ exons intact, and as such, is not expected to have a significant effect on minilocus VDJ recombination.

Analysis of wild-type and EδmCore minilocus VDJ recombination was performed by PCR from thymus genomic DNA templates, using specific Vδ1, Vδ2, jδ1, and jδ3 primers (primers 1, 2, 3, and 4; Fig. 1C) as described previously (13). Previous studies have shown the wild-type minilocus to rearrange stepwise, first V to D, and then VD to J (13). Amplification with Vδ1 and jδ1 primers yields 0.3-kb fragments that represent complete VDJ rearrangements, and in addition, 1.2-kb fragments that represent the VD rearrangement intermediates (Fig. 1D). Amplification with Vδ1 and jδ3 primers yields a 0.3-kb PCR product only. PCR reactions were also performed with a pair of Cδ primers (primers 5 and 6; Fig. 1C) to control for differences in transgene copy number and PCR efficiency between samples; the amount of genomic DNA template used in PCR reactions was typically adjusted to obtain similar Cδ amplification signals in each sample. PCR products were detected and quantified by agarose gel electrophoresis followed by blotting and hybridization with appropriate 32P-labeled probes. In agreement with our previous studies (13), quantification of PCR signals revealed amplification to be linear over a broad range of template concentrations (Fig. 2).

We analyzed the effect of the core site mutation on miniloci.
locus VDJ recombination by comparing VD and VDJ rearrangement levels in thymocytes from three previously characterized lines of mice carrying a minilocus with a wild-type Eδ (A, B, C) (13) to thymocytes in the four lines of mice carrying the EδmCore minilocus (Fig. 3, A and B). All Eδ lines of mice carry single copy integrations of the minilocus. Whereas the minilocus integrations in lines A and B include all relevant gene segments, the integration in line C is truncated at the 5′ end and is missing the Vδ1 gene segment. Thus, lines A and B are informative for both Vδ1 and Vδ2 rearrangement, whereas line C is informative for Vδ2 rearrangement only. As in previous studies, PCR analysis of VDJ recombination in the Eδ lines revealed high levels of 0.3-kb VDJ rearrangement products in each case (Fig. 3, A and B). Levels of 1.2-kb VD rearrangement products were lower and more variable (Fig. 3 A). Thus, as observed previously (13), the enhancer-dependent VD to J step of transgene rearrangement occurs efficiently in each of the Eδ lines.

Analysis of VDJ rearrangement in the four EδmCore lines revealed quite different results. In three of the lines (U, Y1, and Z), 0.3-kb products representing VDJ rearrangement were barely detectable, even though 1.2-kb VD rearrangement products were readily apparent (Fig 3, A and B). In the fourth line (Y2), VD and VDJ rearrangement products were both detected at levels that were comparable to their representation in Eδ lines. The analysis of a second animal in each line (data not shown) yielded quite similar results, arguing that these VDJ recombination phenotypes are stable and reproducible characteristics of the individual transgenic lines. Because PCR amplifications with the Cδ primer pair and with the Vδ1-J1 primer pair were shown to be linear over several orders of magnitude (Fig. 2) we were justified in quantifying the level of VDJ recombination in the different lines by normalizing the Vδ1-J1 PCR signal to the Cδ PCR signal in each line. The levels of VDJ rearrangement in lines U, Y1 and Z, each calculated as the mean of three independent determinations, were found to be 0.8, 1.3, and 0.3%, respectively, of the level in Eδ line A, and 3.1, 4.9, and 1.2%, respectively, of the level in Eδ line B (Table 1). Similar quantification revealed VDJ rearrangement in line Y2 to be 41.7% of the level in line A, and 159.8% of the level in line B (Table 1).

The conclusions drawn from PCR analysis were confirmed through analysis of Vδ1-D3 and Vδ1-D3-J3-J1 rearrangements by genomic Southern blot (Fig. 4). Eδ line A thymocytes displayed nearly undetectable germline Vδ1 (1.0 kb), moderate Vδ1-D3 rearrangement (0.9 kb), and substantial Vδ1-D3-J3-J1 rearrangement (1.7 kb). In accord with the PCR data, Vδ1-D3-J3-J1 rearrangement was not detected in lines U and Z, even though transgene copy was higher in these lines than in Eδ line A. Importantly, Vδ1-D3 rearrangement was readily detected in both lines, and accounted for almost all of the Vδ1 signal in line Z. Although the reduced sensitivity of genomic Southern blot analysis as compared to PCR analysis does not allow an independent evaluation of the extent to which VDJ rearrangement has been specifically inhibited, they provide strong support for the PCR data. Also in accord with the PCR data, Vδ1-D3 and Vδ1-D3-J3-J1 rearrange-
Intact Binding Sites for CBF/PEBP2, c-Myb, and GATA-3

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The V<sub>δ</sub>1-D<sub>δ</sub>3-J<sub>δ</sub>1 and C<sub>δ</sub> hybridization signals were measured in three independent experiments. In each experiment, the V<sub>δ</sub>1-D<sub>δ</sub>3-J<sub>δ</sub>1 signal was normalized to the C<sub>δ</sub> signal, and the results are reported as the mean ± SD. VDJ recombination expressed as a percentage of VDJ recombination in line A.

Table 1. VDJ rearrangement in E<sub>δ</sub>, E<sub>δ</sub>mCore, and δE3,4

| Construct | Line | V<sub>δ</sub>1-D<sub>δ</sub>3-J<sub>δ</sub>1/C<sub>δ</sub> |
|-----------|------|----------------------------------|
| E<sub>δ</sub> | A    | (100)                           |
|           | B    | 26.1 ± 11.1                     |
| E<sub>δ</sub>mCore | U | 0.8 ± 0.4                      |
|           | Y1   | 0.3 ± 0.3                       |
|           | Z    | 1.3 ± 0.8                       |
|           | Y2   | 41.7 ± 7.8                      |
| δE3,4     | JA   | 1.4 ± 0.6                       |
|           | JG   | 4.7 ± 2.6                       |

The V<sub>δ</sub>1-D<sub>δ</sub>3-J<sub>δ</sub>1 and C<sub>δ</sub> hybridization signals were measured in three independent experiments. In each experiment, the V<sub>δ</sub>1-D<sub>δ</sub>3-J<sub>δ</sub>1 signal was normalized to the C<sub>δ</sub> signal, and the results are reported as the mean ± SD. VDJ recombination expressed as a percentage of VDJ recombination in line A.

These results are reminiscent of our previous work analyzing VDJ recombination within a TCR-δ E<sub>δ</sub> minilocus lacking C<sub>δ</sub> (13) or containing a disrupted binding site for C<sub>δ</sub>(39). In four of five transgenic lines carrying the minilocus construct lacking C<sub>δ</sub>, and in three of four transgenic lines carrying the minilocus construct with a disrupted binding site for C<sub>δ</sub>, inhibition of the VD to J rearrangement step was almost complete. However, in each case one of the transgenic lines displayed higher levels of VD to J rearrangement. Such heterogeneity in TCR-δ minilocus transgenic lines in which E<sub>δ</sub> has been eliminated or inactivated probably reflects the distinct properties of the different transgene integration sites. It is proposed that in E<sub>δ</sub>, E<sub>δ</sub>mCore, and E<sub>δ</sub>-mCore lines in which the VD to J step still occurs, the minilocus has integrated adjacent to active regulatory elements that partially or completely supplant the need for E<sub>δ</sub>. Heterogeneity of this magnitude has not been observed in transgenic lines carrying an intact enhancer, as VD to J rearrangement is efficient in three of three E<sub>δ</sub> lines (Fig. 3) and four of four E<sub>δ</sub> lines (14). On the basis of the phenotype displayed by the majority of E<sub>δ</sub>mCore transgenic lines, we conclude that elimination of a functional CBF/PEBP2 binding site within E<sub>δ</sub> has a dramatic effect on the ability of E<sub>δ</sub> to provide the regional accessibility to the VDJ recombinase that is required for efficient VDJ recombination in vivo.

Intact Binding Sites for CBF/PEBP2, c-Myb, and GATA-3 are not Sufficient for Efficient TCR-δ Gene Rearrangement in Vivo. Previous in vitro binding and transient transfection studies identified a functionally important binding site for c-Myb that is within the δE3 element and adjacent to the CBF/PEBP2 binding site (27), as well as two functionally important binding sites for GATA-3 within the adjacent δE4 element (40, 41). A 60-bp δE3,4 fragment of E<sub>δ</sub> containing only these binding sites displays 20–50% of the activity of the intact 1.4-kb E<sub>δ</sub> in transient transfection experiments (25). Since CBF/PEBP2 (this study) and c-Myb (39) were implicated as important regulators of VDJ recombination in vivo, we asked whether the combination of CBF/PEBP2, c-Myb and GATA-3 binding sites within δE3 and δE4 was sufficient to activate this process.

Therefore, the 60-bp δE3,4 fragment of E<sub>δ</sub> was introduced into the TCR-δ gene minilocus in place of E<sub>δ</sub> (Fig. 1B). Three transgenic founders were obtained and were used to generate independent lines of transgenic mice designated JA, JE, and JG. Slot blot analysis indicated transgene copy numbers of 2, 1, and 24 for JA, JE, and JG, respectively. As in E<sub>δ</sub> line C, the single copy δE3,4 line JE is truncated such that it lacks V<sub>δ</sub>1 but retains V<sub>δ</sub>2. Hence, this line is informative for V<sub>δ</sub>2 rearrangement only.

Analysis of wild-type E<sub>δ</sub> and δE3,4 minilocus VDJ recombination was performed by PCR as described above. All three δE3,4 lines revealed dramatically reduced levels of VDJ rearrangement as compared to E<sub>δ</sub> lines A, B, and C (Fig. 5, A and B). In lines JA and JE, VDJ rearrangement was essentially undetectable, whereas in line JG, VDJ rearrangement was detectable at reduced levels. Nevertheless, VDJ rearrangement signals were readily detected by PCR in all three lines, and were detected at particularly high levels in line JA. Quantification of V<sub>δ</sub>1-D<sub>δ</sub>3 and C<sub>δ</sub> PCR signals indicated that VDJ rearrangement in lines JA and JG were reduced to 1.4 and 4.7%, respectively, of the level in E<sub>δ</sub> line A, and to 5.2 and 17.9%, respectively, of the level in E<sub>δ</sub> line B (Table 1). Analysis of V<sub>δ</sub>1-D<sub>δ</sub>3 and V<sub>δ</sub>1-D<sub>δ</sub>3-J<sub>δ</sub>1 rearrangements by genomic Southern blot confirmed that essentially all copies of V<sub>δ</sub>1 were rearranged to D<sub>δ</sub>3 in line JA, but that rearrangement was blocked at this stage (Fig. 4).
Although the low level of VDJ rearrangement detected in line JG could not be confirmed due to both the limited sensitivity of detection and the presence of comigrating germline fragments in the tail DNA control, the results for JG did suggest that PCR may have underestimated the level of V1-J3 rearrangement in this line. Also of note is the significant reduction in transgene copy number in thymus relative to tail in this line (Fig. 4 and data not shown). This most likely results from the rearrangement of V1-J3 in one copy of the tandemly arrayed transgene to D2 in another copy, with deletion of intervening copies. In summary, on the basis of the dramatic inhibition of VD to J recombination detected in both PCR and genomic Southern blot analyses, we conclude that the combination of CBF/PEBP2 and c-Myb, and GATA-3 binding sites contained within δE3 and δE4 is not by itself capable of promoting the accessible chromatin configuration required for efficient minilocus VDJ recombination.

Discussion

We previously documented enhancer-dependent VDJ recombination within a human TCR-δ gene minilocus construct in transgenic mice (13, 14), and have now begun to address the mechanisms by which Eδ exerts its effects on VDJ recombination in this system. The data presented here indicates that a mutation that destroys the binding site for the transcription factor CBF/PEBP2 within the δE3 element of Eδ seriously compromises the ability of Eδ to activate the VD to J step of minilocus rearrangement. Hence, by binding to Eδ, this or a very closely related factor plays a crucial role in the developmental activation of minilocus rearrangement in vivo. We interpret the pattern of transgene rearrangement in the presence or absence of a functional Eδ to indicate that a functional Eδ is required to promote the accessibility of Jδ gene segments to the VDJ recombinase within the transgenic minilocus. Although not directly proven, we infer that a functional Eδ is also required to promote Jδ gene segment accessibility, and hence VDJ recombination, within the endogenous TCR-δ locus. Our data therefore suggest strongly that CBF/PEBP2 family transcription factors are likely to be important regulators of TCR-δ gene rearrangement at the endogenous TCR-δ locus in vivo. Nevertheless, formal proof for this notion would require elimination of the Eδ CBF/PEBP2 binding site from the endogenous locus by homologous recombination.

CBF/PEBP2 was initially identified by virtue of its ability to bind to and activate transcription from polyoma virus enhancer and the long terminal repeats of murine T lymphotropic retroviruses (42, 43). Functional CBF/PEBP2 binding sites have been identified in the regulatory elements of several cellular genes expressed in either the T lymphoid or myeloid cell lineages (44–48), including the enhancers of all four TCR genes (30, 33–36). CBF/PEBP2 is actually a complex family of transcription factors, each of which is composed of a DNA-binding α subunit and an associated β subunit (29–31). Three distinct genes (αA, αB, and αC) encode related α subunits (30, 49–52), a separate gene encodes a shared β subunit (29, 31), and additional complexity is introduced by production of multiple α and β isoforms from the individual genes (29–31, 53). Thus, any one of a number of CBF/PEBP2 species might be the crucial regulator of TCR-δ gene rearrangement in vivo. Although recent analysis of CBF/PEBP2 αB null mice emphasizes a crucial role for this particular factor in hematopoiesis, the early lethality and pleiotropic effects of this mutation preclude any specific conclusions regarding a role in TCR-δ gene rearrangement (37). A candidate regulator of TCR-δ gene rearrangement would have to be expressed as early as the CD4+CD8− stage of thymocyte development, since the endogenous TCR-δ locus (54, 55) and our transgenic minilocus (14) are both activated during this stage. Notably, CBF/PEBP2 αA and αB are both expressed at highest levels in the thymus and are both expressed in CD4+CD8− thymocytes (56). Thus, both of these factors have expression patterns that would be consistent with a role in the activation TCR-δ gene rearrangement and expression in vivo. Related studies using the transgenic minilocus approach have also implicated the transcription factor c-Myc in the activation of TCR-δ gene rearrangement in vivo (39). Hence CBF/PEBP2 and c-Myc appear to synergize to activate TCR-δ gene VDJ recombination in vivo, much as they were found to synergistically activate TCR-δ gene transcription.
in transient transfection studies (27, 28). Nevertheless, our results indicate that the combination of CBF/PEBP2, Myb, and GATA-3 binding sites within δE3 and δE4 is not, by itself, sufficient to promote the accessibility required for efficient activation of VDJ recombination within the TCR-δ gene minilocus. This suggests that additional δs-acting elements contained within the 1.4-kb Eδ are crucial for this process. Such elements may be contained within the 370-bp fragment of Eδ found to contain maximal enhancer activity in transient transfection experiments. Analyses of truncated forms of the enhancer suggest δE2, δE5, and δE6 as candidate δs-acting elements of Eδ that might individually or in combination increase the activity of the minimal enhancer by two- to threefold (24, 25). Little is known about the identities of the factors that interact with these elements. Nevertheless, it may be misleading to identify candidate determinants of Eδ recombinational enhancer activity in a chromosomally integrated context in transgenic mice by extrapolating from those required for transcriptional enhancer activity in transient transfection experiments. For example, nuclear matrix attachment sites that flank Eδ are irrelevant for transcriptional activity as measured in transient transfection experiments or in chromosomally integrated substrates in stably transfected cells, but are important for the induction of transcriptional activity and general sensitivity to DNase I digestion in a chromosomally integrated substrate in transgenic mice (57). Similarly sequences that flank the human adenosine deaminase gene enhancer are irrelevant for transcriptional activity in transient transfection experiments, but are required for high level expression and the establishment of enhancer DNase I hypersensitivity in transgenic mice (58, 59). It is therefore quite possible that δs-elements contained within the 1.4-kb Eδ might not appear relevant for gene expression on the basis of transient transfection experiments, but might be critical for Eδ induced accessibility and VDJ recombination in transgenic mice. These additional δs-acting elements could be required for the stable assembly of CBF/PEBP2, c-Myb, and GATA-3 onto their δE3 and δE4 binding sites in a chromatin context. Alternatively, δE3 and δE4 might by themselves be able to support the assembly of a stable nucleoprotein complex, but additional δs-acting enhancer elements might contribute independently to accessibility and VDJ recombination. The hierarchy of assembly of nucleoprotein complexes at Eδ, and the mechanisms by which assembled nucleoprotein complexes modulate regional chromatin accessibility and VDJ recombination, will be important issues to address in future studies.

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