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The Immunoglobulin Heavy-Chain Enhancer Functions as the Promoter for Iμ Sterile Transcription

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We demonstrate that the immunoglobulin heavy-chain enhancer functions as the promoter for Iμ sterile transcription. The enhancer itself, when placed 5′ to the bacterial *cat* or *neo* genes, is able to direct transcription by using heterogeneous start sites that are generally the same as those found with bona fide Iμ transcripts. In general, promoter activity is dependent on the same sequence motifs important for enhancer activity. However, it appears that a mutation within the conserved octanucleotide ATTTGCAT has a much more severe effect on the promoter activity of the enhancer than the same mutation has on its enhancer activity. This result is consistent with the known role of the octanucleotide as a promoter element, and this is discussed in relation to the biological role of sterile transcription.

Transcription within the immunoglobulin heavy-chain (IgH) locus in B lymphocytes consists primarily of two classes of transcription units. One class results from the transcription of rearranged genes, initiating at the V (variable) region promoter and proceeding through the V, D (diversity), J (joining), and C (constant) regions (for a review, see reference 37). This transcription is thought to be stimulated by the IgH enhancer located in the intron between the J and C regions (3, 9, 25) and, in the case of productively rearranged genes, it leads to the synthesis of heavy-chain protein. A second class includes the "sterile transcripts"; for convenience, this nomenclature will refer to transcripts that have an inherent inability to encode functional antibody proteins. One type of sterile transcript originates from the V region promoters of unrearranged genes and is detected only during early fetal development (36). It is not known how these transcripts arise, since the promoters of unrearranged V regions are not known to be linked to enhancers. Another type of sterile transcript originates either within the introns that immediately precede the constant regions (13, 18, 24, 33, 34, 38) or 5′ to some D-J-joined regions (31). Certain sterile transcripts can be observed throughout B-cell development (13). It has been postulated that the presence of sterile transcripts reflects (or establishes) a form of chromatin that is permissive for the initial rearrangement of the genes and for subsequent class switching (5, 18, 33, 34, 38). Hence, it is possible that sterile transcription plays a pivotal role in B-cell development.

The Iμ transcripts are sterile transcripts whose start sites are heterogeneous and map to the vicinity of the IgH enhancer within the J-C intron (13, 17, 24). It has been proposed that these transcripts arise as a consequence of transcriptional elements within the enhancer (17). In particular, the conserved octanucleotide (ATTTGCAT) within the enhancer falls in a position relative to the start sites that approximates its other locations within the V region promoters of both heavy- and kappa light-chain genes (7, 27). The heterogeneous 5′ ends of the Iμ transcripts may reflect the fact that, unlike the V region promoters, there is no apparent TATA element 3′ to the octanucleotide within the enhancer.

Here we demonstrate that the IgH enhancer does, in fact, directly serve as the promoter for Iμ transcripts. Furthermore, by testing a variety of enhancer mutants, we have defined the enhancer motifs important for this activity. Our results indicate that the octanucleotide is much more critical as a promoter element than it is as an enhancer element.

MATERIALS AND METHODS

Plasmids. All plasmids were constructed by using standard techniques (20). Plasmids A and B (Fig. 1) were derived from plasmids carrying IgH enhancer fragments 1 and 16, respectively (14). Those fragments were isolated by first digesting with *Taq*I, and the resulting 5′ overhangs were then blunted by treatment with deoxynucleoside triphosphates and the large fragment of *Escherichia coli* DNA polymerase. After subsequent digestion with *Bam*HI, the *Bam*HI-*Taq*I fragments were used to substitute the β-globin promoter fragment (*Bam*HI to *Hin*dIII) in the plasmid pSVAβGcat(X) (12) with the *Hin*dIII site of the plasmid being blunted (as described above). Plasmids carrying the simian virus 40 (SV40) enhancer (A-SV and B-SV; Fig. 1) were derived from pSVAβGcat-XLRS, a plasmid carrying the S40 enhancer 3′ to the *cat* gene. The pSVAβGcat-XLRS plasmid was constructed as follows. The SV40 enhancer was isolated from pSV232Aneo-LR (11) as a *Bam*HI fragment and was inserted into the *Bam*HI site of M13mp18, and the orientation of the inserts was determined by DNA sequencing. The enhancer was then removed from M13mp18 as an *Eco*RI-to-*Sal*I fragment and inserted into the region between the *Sal*I and *Eco*RI sites 3′ to the *cat* gene in pSVAβGcat(X). The orientation of the SV40 enhancer in these plasmids is the same as that of the β-globin promoter. All plasmids carrying the *neo* gene (32) in place of the *cat* gene have the SV40 enhancer situated 3′ to the *neo* gene. Plasmids carrying mutant IgH enhancers 5′ to the *cat* gene were constructed generally as described above by using the mutant enhancer fragments described previously (14). Plasmids used for the

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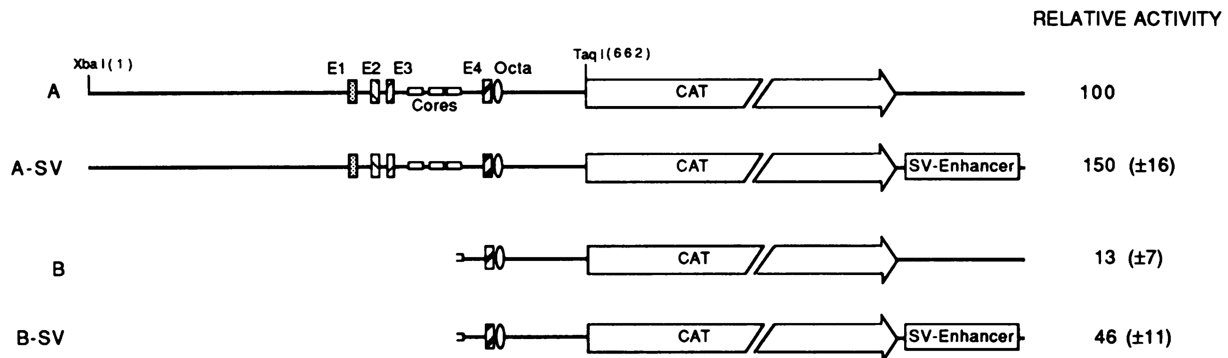

FIG. 1. Relative promoter activities of IgH enhancer fragments. Transient transfections of P3-X63Ag8 cells and *cat* assays were carried out as described in the text. The enhancer sequence motifs (μE1, μE2, μE3, the core sequences, μE4, and the octanucleotide [Octa]) are indicated. Relative promoter activities were derived from five separate transfections after subtracting values obtained with the promoterless plasmid pSV0Acat.

generation of riboprobes were all derived from pGEM3 (Promega Biotec). One of these plasmids contains the *Xba*I-to-*Eco*RI fragment of the IgH enhancer and was used to generate the riboprobe for mapping endogenous Iμ transcripts. A second plasmid contains a *Xba*I-to-*Dde*I fragment with both enhancer and *neo* gene sequences. The enhancer sequences in this second plasmid extend from a mutant μE4 site (made into a *Xba*I site at position 529 [14]) to the *Taq*I site, and the *neo* sequences extend from the *Hin*dIII site to the *Dde*I site within the *neo* gene at +24 (the *Taq*I and *Hin*dIII sites were linked as described for the plasmids outlined above). The *Dde*I site was blunted, and the fragment was inserted into pGEM3 between *Xba*I and a blunted *Hin*dIII site. This plasmid was used to generate riboprobes for mapping the transcripts from transfected chimeric enhancer-*neo* genes.

Cell lines and transfections. Mouse cell lines P3-X63Ag8 (15) and J558L (26) were grown in Dulbecco minimal essential medium containing 10% fetal bovine serum. P3-X63Ag8 and J558L cells were transfected by electroporation (30) as follows. Cells were suspended in 1X Hanks balanced salt solution (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid], 140 mM NaCl, 1.5 mM Na_2HPO_4 [pH 7.05]) at a density of 10^7 cells per ml. One milliliter of suspended cells was mixed with 20 to 50 μg of linearized plasmid and then subjected to an electric discharge of 500 V/cm for P3-X63Ag8 cells or 625 V/cm for J558L cells at room temperature (both at 1,080 μF). For transient assays, cells were harvested 2 days after transfection. Stable transformants containing the *neo* plasmids were transfected as described above and then selected in medium containing G418 (GIBCO Laboratories) at the effective concentrations of 0.3 mg/ml for P3-X63Ag8 cells and 0.5 mg/ml for J558L cells. Selective medium was applied 2 days after transfection.

CAT assays and RNase protection assays. Chloramphenicol acetyltransferase (CAT) assays were carried out essentially as described by Gorman et al. (10). For each transfection experiment, promoter activity was determined after subtracting CAT levels obtained with cells transfected with the plasmid pSV0Acat (constructed from pSV0Adhfr; 11), which carries no promoter 5' to the *cat* gene. Poly(A)$^+$ RNA was isolated as described by Badley et al. (1). Preparation of RNA probes (see pGEM plasmids, as described above) and RNase protection assays were carried out by the methods of Melton et al. (23) and Zinn et al. (39). Hybridizations were performed at 40°C overnight and RNase digestion was carried out at 20°C for 2 h. Protected probe fragments were resolved on 10% polyacrylamide–urea gels.

RESULTS

To determine whether the IgH enhancer possesses promoter activity, we constructed a series of plasmids in which segments of the enhancer were placed immediately 5' to the bacterial *cat* gene (10). Initially, two different enhancer fragments were tested, each in the presence or absence of the SV40 enhancer (Fig. 1). One fragment extends from the *Xba*I site (position 1) to the *Taq*I site (position 662) by the numbering system of Gillies et al. (9). The other extends from the third enhancer core element (modified by mutation into an *Xba*I site at position 473 [12]) to the *Taq*I site. This second fragment corresponds to what we previously described as enhancer domain B (14). The *Taq*I site was chosen specifically to avoid an enhancer-encoded AUG being incorporated into the mRNA to thus ensure proper translational initiation of the *cat* RNA. Plasmids were transiently transfected into the mouse plasmacytoma cell lines P3-X63Ag8 and J558L, and CAT activity was measured in crude extracts prepared 2 days later. As a negative control, a plasmid containing no potential promoter sequences was also transfected.

The intact enhancer does indeed drive expression of the *cat* gene (Fig. 1, plasmid A). The levels were approximately 5% of those we obtained with an SV40 early promoter (pSV2cat [10]; data not shown). The overall promoter activity was slightly increased (to 150%) by adding the SV40 enhancer 3' to the *cat* gene (plasmid A-SV) and was dramatically reduced (to 13%) when only enhancer domain B was used to drive *cat* expression (plasmid B) (Fig. 1). This latter activity can be stimulated roughly fourfold by adding the SV40 3' enhancer (plasmid B-SV). Similar results were obtained with J558L cells (data not shown). It is known that enhancer domain B lacks several positive-acting enhancer motifs (e.g., μE1, μE2, and μE3; 14, 16, 28, 35), so it is not surprising that this domain has weaker promoter activity and that this activity is stimulated by the SV40 enhancer.

To determine whether the enhancer is indeed acting as the promoter for the production of Iμ transcripts, we mapped the 5' ends of the enhancer-promoted transcripts and compared these directly with the 5' ends of bona fide Iμ transcripts. This mapping was fortuitously simplified by the

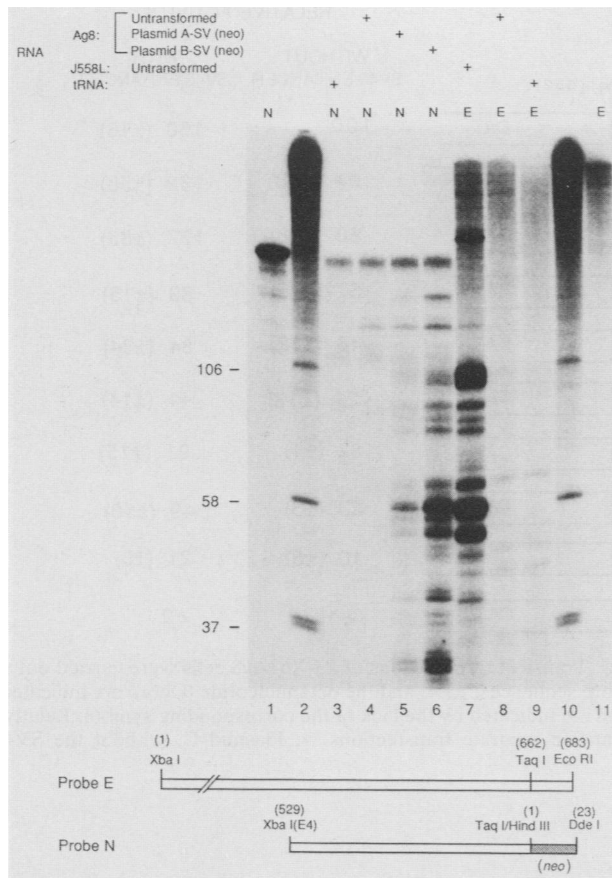

FIG. 2. Transcription start sites within transfected and endogenous IgH enhancers. P3-X63Ag8 cells (Ag8) were stably transformed with plasmids similar to A-SV and B-SV (Fig. 1), except that the *neo* gene was used in place of the *cat* gene. PolyA$^+$ RNA was isolated from untransformed J558L cells, and P3-X63Ag8 cells were stably transformed with each plasmid. The sources of RNA used in each RNase protection assay are indicated at the top left of the figure, with corresponding signs above each lane. The probes used (N or E) are also indicated above each lane and are shown below the autoradiogram. Lane 1, Undigested probe N alone; lanes 2 and 10, RNA size markers (nucleotide lengths are indicated at left); lanes 3 through 6, probe N incubated with, respectively, tRNA, RNA from untransformed Ag8 cells, RNA from Ag8 cells transformed with plasmid A-SV(neo), and RNA from Ag8 cells transformed with plasmid B-SV(neo); lanes 7 through 9, probe E incubated with, respectively, RNA from untransformed J558L cells, RNA from untransformed Ag8 cells, and tRNA; lane 11, undigested probe E alone.

fact that our particular line of P3-X63Ag8 cells does not itself produce Iμ transcripts. We determined by Southern analysis that this is due to a deletion of enhancer DNA within the nontranslocated IgH locus in these cells (data not shown). We suspect that the deletion extensively disrupted what was once a productive heavy-chain locus because we could not detect immunoglobulin in the medium of these particular cells. We used these cells to make stable transformants with plasmids analogous to A-SV and B-SV (Fig. 1), except that the *cat* gene was replaced with the *neo* gene. Transformants expressing *neo* were selected in G418.

An RNase protection assay that maps RNAs complimentary to the IgH enhancer is shown in Fig. 2. RNA was isolated from J558L cells (Fig. 2, lane 7), untransformed P3-X63Ag8 cells (lanes 4 and 8), and P3-X63Ag8 cells transformed with the plasmids carrying the IgH enhancer fragments 5′ to the *neo* gene (lanes 5 and 6). The results demonstrate that most of the 5′ ends derived from each of the two plasmids transfected into P3-X63Ag8 cells are identical to one another and correspond very well to those seen within the endogenous enhancer that resides in J558L cells (compare lanes 5 and 6 with lane 7; the slight differences in apparent mobility are due to a difference of one nucleotide in the protected regions of the two different probes used). It is possible that some of the moderate differences in relative band intensities (e.g., note bands at 100 nucleotides) are due to differences between the P3-X63Ag8 and J558L cell lines. This type of cell line-specific difference for Iμ transcripts has been noted previously (17). The major differences in 5′ ends map approximately to positions 530 and 645 in the enhancer (protecting fragments of approximately 130 and 35 nucleotides, respectively) which are seen only with the transfected plasmids. At present we have no explanation for these apparently new transcription start sites, although they may also reflect cell line-specific differences. It is also possible that the start site that maps to position 645 is somehow facilitated by the *neo* sequences that reside only 15 nucleotides away. We have noted that the major 5′ ends seen in our cell lines correspond well to those previously reported for Iμ transcripts in other cell lines (17). We conclude from these results that the enhancer alone is sufficient to drive the synthesis of sterile Iμ transcripts.

To investigate the *cis* elements required for the promoter activity of the enhancer, we examined the effects of mutating the E motifs (μE1, μE2, μE3, and μE4) and the octanucleotide. The plasmids used and the results obtained from transient transfections of P3-X63Ag8 cells are shown in Fig. 3 (similar results were obtained with J558L cells [data not shown]). Because of the way these plasmids were constructed, all of these enhancers also carry a mutation within the third enhancer core element. However, there is no detectable effect of this mutation (compare plasmids A and C). With the possible exception of the μE1 element, mutations in the E motifs reduced the overall promoter activity of the enhancer. However, mutation of any single E motif reduced the promoter activity no more than twofold, and, with the exception of the μE4 motif (see Discussion), the results in this regard are similar to those from experiments in which these same mutations were tested for their effects on enhancer activity per se (14). The activity of the μE2-μE3 double mutation was approximately the same as that of domain B alone (Fig. 1). Mutation of the octanucleotide had the most deleterious effect among all single motif mutations, reducing the promoter activity about fourfold. Deletion of both μE4 and the octanucleotide (and sequences in between) further decreased the promoter activity to only 10% of that of the wild type. Transcription initiation sites of most of the mutant promoters were mapped by using plasmids carrying the *neo* gene as described above. The initiation sites from the mutant promoters tested showed no significant change (Fig. 4); the faster-migrating band at approximately 120 nucleotides (Fig. 4, lane 9) can be explained possibly by mutations introduced into the octanucleotide element being incorporated into RNA and therefore resulting in a specific discontinuity with the nonmutant RNA probe.

DISCUSSION

The studies of Lennon and Perry (17) proposed that sterile Iμ transcripts result from transcription elements within the IgH enhancer. We have demonstrated here that the IgH

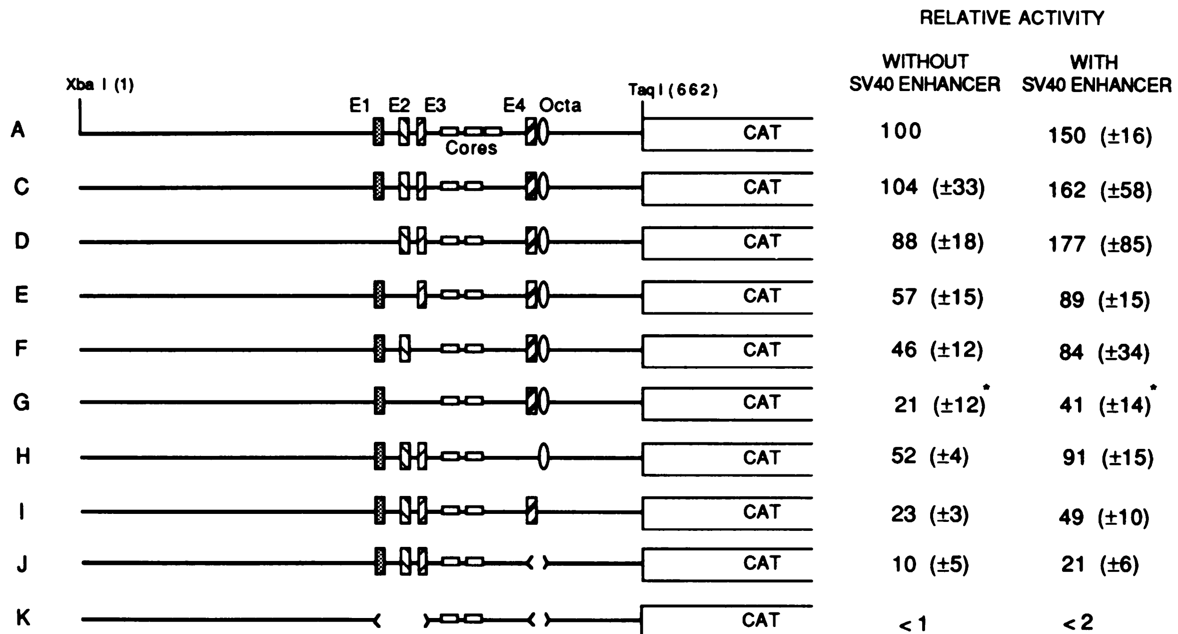

FIG. 3. Mutational analysis of the promoter activity of the IgH enhancer. Transient transfections of P3-X63Ag8 cells were carried out as described in the text. The enhancer sequence motifs (μE1, μE2, μE3, the core sequences, μE4, and the octanucleotide [Octa]) are indicated. Mutations of these motifs are identical to those described previously (14) and are indicated by the lack of the corresponding symbol. Relative promoter activities (means ± the standard deviations) were derived from five separate transfections. *, Plasmid C, without the SV40 enhancer, was used to calculate relative promoter activities.

enhancer functions as the Iμ promoter per se on the basis of its ability to direct the transcription of linked genes by employing the authentic Iμ initiation sites. The promoter activity of the enhancer can be divided into a basal element (consisting of μE4 and the octanucleotide) and an upstream stimulating element (consisting of at least the μE2 and μE3 motifs).

These results are consistent with our previous suggestion that the composition of the enhancer as two functional domains may reflect a dual role of this element as both a promoter and an enhancer (14). Although the μE1, μE2, and μE3 motifs appear to be about equally important for enhancer and promoter function, the μE4 and octanucleotide motifs appear to be much more important for promoter activity. Our μE4 and octanucleotide mutations decrease overall enhancer activity less than 10 and 20%, respectively (14), while these same mutations reduce promoter activity two- and fourfold, respectively. In addition, in contrast to a very slight effect on enhancer activity (14), simultaneous deletion of μE4 and the octanucleotide motifs almost completely abolishes promoter activity. The finding that the octanucleotide is very important for the promoter activity of the enhancer is not surprising, since it has been shown to be essential for both IgH and Igκ promoter activities (2, 6, 7, 22, 27). What may be more surprising is that the octanucleotide can, be itself, function at a distance as an enhancer element (8). It is becoming increasingly clear that this is a property of some, but not all, transcription elements (4, 21). Unlike the octanucleotide, the μE4 motif does not have an obvious counterpart in either heavy- or light-chain promoters. A conserved heptanucleotide which is found adjacent (5′) to the octanucleotide in a variety of immunoglobulin promoters has been shown to be important for promoter activity (2, 6). Interestingly, this element has been shown to stimulate cooperative binding of the OCT-2 protein to adjacent heptamer and octamer sites, even though it bears no sequence similarity to the octanucleotide (29). Although binding of proteins (including OCT-1 and OCT-2) to the μE4 motif has not been observed in vitro (8), its position adjacent (5′) to the octanucleotide and its important role as a promoter element raise the possibility of a parallel relationship with the heptamer.

It has been suggested that sterile transcription is a prerequisite for DNA rearrangement events within both heavy- and light-chain immunoglobulin loci. A variety of correlative observations and direct experiments support this. Alt and co-workers have demonstrated with plasmid substrates that recombination efficiency correlates with transcription activity (5); they have also shown that certain unrearranged V regions are transcriptionally active at a specific stage during early fetal development (36). Moreover, a number of reports indicate that class switching correlates with the presence of very specific sterile transcripts (19, 33, 34, 37). Finally, Lennon showed that Iμ transcripts can be detected in fetal liver before appreciable transcription of rearranged heavy-chain genes has taken place (Ph.D. thesis, University of Pennsylvania, Philadelphia, 1988). Taken together, these results lead to a model in which transcription itself renders chromatin accessible to the recombination machinery. Our results are consistent with the general notion that at least Iμ transcription is important. The composition of the enhancer supports the idea that promoter activity has been specifically designed into its structure. Again, this is most clearly demonstrated by the position of the octanucleotide which, by our assays, is not particularly important for overall enhancer function but is crucial for Iμ transcription.

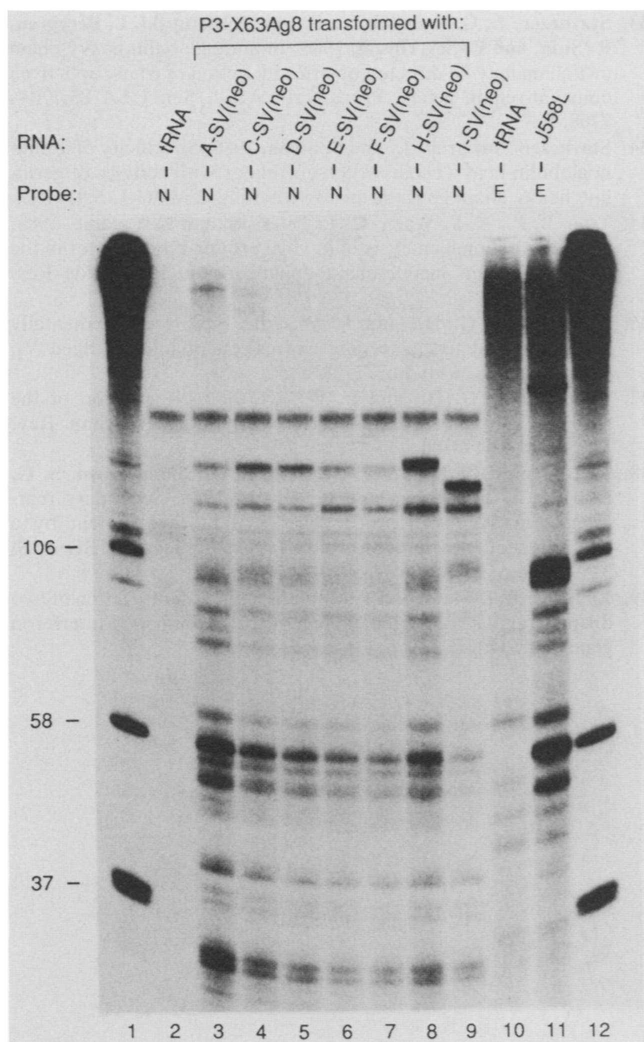

FIG. 4. Transcription start sites within mutant enhancers. P3-X63Ag8 cells were stably transformed with the indicated plasmids carrying the *neo* gene instead of the *cat* gene. Probes used in the analysis are shown in Fig. 2.

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LITERATURE CITED

1. **Badley, J. E., G. A. Bishop, T. St. John, and J. A. Frelinger.** 1988. A simple, rapid method for the purification of poly A^+ RNA. Biotechniques **6:**114–116.
2. **Ballard, D. W., and A. Bothwell.** 1986. Mutational analysis of the immunoglobulin heavy chain region. Proc. Natl. Acad. Sci. USA **83:**9626–9630.
3. **Banerji, J., 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.
4. **Bienz, M., and H. R. B. Pelham.** 1986. Heat shock regulatory elements function as an inducible enhancer in the Xenopus hsp70 gene and when linked to a heterologous promoter. Cell **45:**753–760.
5. **Blackwell, T. K., M. W. Moore, G. D. Yancopoulos, H. Suh, S. Lutzker, E. Selsing, and F. W. Alt.** 1986. Recombination between immunoglobulin variable region gene segments is enhanced by transcription. Nature (London) **324:**585–589.
6. **Eaton, S., and K. Calame.** 1987. Multiple DNA sequence elements are necessary for the function of an immunoglobulin heavy chain promoter. Proc. Natl. Acad. Sci. USA **84:**7634–7638.
7. **Falkner, F. G., and H. G. Zachau.** 1984. Correct transcription of an immunoglobulin κ gene requires an upstream fragment containing conserved sequence elements. Nature (London) **310:**71–74.
8. **Gerster, T., P. Matthias, M. Thali, J. Jiricny, and W. Schaffner.** 1987. Cell type-specificity elements of the immunoglobulin heavy chain gene enhancer. EMBO J. **6:**1323–1330.
9. **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.
10. **Gorman, G. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
11. **Kadesch, T., and P. Berg.** 1986. Effects of the position of the simian virus 40 enhancer on expression of multiple transcription units in a single plasmid. Mol. Cell. Biol. **6:**2593–2601.
12. **Kadesch, T., P. Zervos, and D. Ruezinsky.** 1986. Functional analysis of the murine IgH enhancer: evidence for negative control of cell-type specificity. Nucleic Acids Res. **14:**8209–8221.
13. **Kemp, D. J., A. W. Harris, and J. M. Adams.** 1980. Transcription of the immunoglobulin Cμ gene varies in structure and splicing during lymphoid development. Proc. Natl. Acad. Sci. USA **77:**7400–7404.
14. **Kiledjian, M., L.-K. Su, and T. Kadesch.** 1988. Identification and characterization of two functional domains within the murine heavy-chain enhancer. Mol. Cell. Biol. **8:**145–152.
15. **Kohler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256:**495–497.
16. **Lenardo, M., J. W. Pierce, and D. Baltimore.** 1987. Protein-binding sites in Ig gene enhancers determine transcriptional activity and inducibility. Science **236:**1573–1577.
17. **Lennon, G. G., and R. P. Perry.** 1985. Cμ-containing transcripts initiate heterogeneously within the IgH enhancer region and contain a novel 5'-nontranslatable exon. Nature (London) **318:**475–478.
18. **Lutzker, S., and F. W. Alt.** 1988. Structure and expression of germ line immunoglobulin γ2b transcripts. Mol. Cell. Biol. **8:**1849–1852.
19. **Lutzker, S., P. Rothman, R. Pollock, R. Coffman, and F. W. Alt.** 1988. Mitogen- and IL-4-regulated expression of germ-line Ig γ2b transcripts: evidence for directed heavy chain class switching. Cell **53:**177–184.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Maniatis, T., S. Goodbourn, and J. A. Fischer.** 1987. Regulation of inducible and tissue-specific gene expression. Science **236:**1237–1245.
22. **Mason, J. O., G. T. Williams, and M. S. Neuberger.** 1985. Transcription cell type specificity is conferred by an immunoglobulin V_H promoter that includes a functional consensus sequence. Cell **41:**479–487.
23. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **18:**7035–7057.
24. **Nelson, K. J., J. Haimovich, and R. P. Perry.** 1983. Characterization of productive and sterile transcripts from the immunoglobulin heavy-chain locus: processing of μ_m and μ_s mRNA. Mol. Cell. Biol. **3:**1317–1332.
25. **Neuberger, M.** 1983. Expression and regulation of an immuno-

globulin heavy chain gene transfected into lymphoid cells. EMBO J. 2:1373–1378.

26. **Oi, V. T., S. L. Morrison, L. A. Herzenberg, and P. Berg.** 1983. Immunoglobulin gene expression in transformed lymphoid cells. Proc. Natl. Acad. Sci. USA **80:**825–829.
27. **Parslow, T. G., D. L. Blair, W. J. Murphy, and D. K. Granner.** 1984. Structure of the 5′ ends of immunoglobulin genes: a novel conserved sequence. Proc. Natl. Acad. Sci. USA **81:**2650–2654.
28. **Perez-Mutul, J., M. Macchi, and B. Wasylyk.** 1988. Mutational analysis of the contribution of sequence motifs within the IgH enhancer to tissue specific transcriptional activation. Nucleic Acids Res. **13:**6085–6096.
29. **Poellinger, L., B. K. Yoza, and R. G. Roeder.** 1989. Functional cooperativity between protein molecules bound at two distinct sequence elements of the immunoglobulin heavy-chain promoter. Nature (London) **337:**573–576.
30. **Potter, H., L. Weir, and P. Leder.** 1984. Enhancer-dependent expression of human κ immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. Proc. Natl. Acad. Sci. USA **81:**7161–7165.
31. **Reth, M. G., and F. W. Alt.** 1984. Novel immunoglobulin heavy chains are produced from DJμ gene segment rearrangements in lymphoid cells. Nature (London) **312:**418–423.
32. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1:**327–341.
33. **Stavnezer, J., G. Radcliffe, Y.-C. Lin, J. Nietupski, L. Berggren, R. Sitia, and E. Severinson.** 1988. Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. Proc. Natl. Acad. Sci. USA **85:**7704–7708.
34. **Stavnezer-Nordgren, J., and S. Sirlin.** 1986. Specificity of immunoglobulin heavy chain switch correlates with activity of germline heavy chain genes prior to switching. EMBO J. **5:**95–102.
35. **Tsao, B. P., X.-F. Wang, C. L. Peterson, and K. Calame.** 1988. In vivo functional analysis of in vitro protein binding sites in the immunoglobulin heavy chain enhancer. Nucleic Acids Res. **16:**3239–3253.
36. **Yancopoulos, G. D., and F. W. Alt.** 1985. Developmentally controlled and tissue-specific expression of unrearranged V_H gene segments. Cell **40:**271–281.
37. **Yancopoulos, G. D., and F. W. Alt.** 1986. Regulation of the assembly and expression of variable-region genes. Annu. Rev. Immunol. **4:**339–368.
38. **Yancopoulos, G. D., R. A. DePinho, K. A. Zimmerman, S. G. Lutzker, N. Rosenberg, and F. W. Alt.** 1986. Secondary rearrangement events in pre-B cells: V_HDJ_H replacement by a LINE-1 sequence and directed class-switching. EMBO J. **5:**3259–3266.
39. **Zinn, K., D. DiMaio, and T. Maniatis.** 1983. Identification of two distinct regulatory regions adjacent to the human β-interferon gene. Cell **34:**865–879.