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Complex Regulation of the Immunoglobulin μ Heavy-Chain Gene Enhancer: μB, a New Determinant of Enhancer Function

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The B-lymphocyte-specific activity of the immunoglobulin μ heavy-chain gene enhancer has been attributed to the octamer motif (ATTTGCAT) present within the enhancer that binds a B-cell-specific factor designated NF-A2/OTF-2. However, significant residual enhancer activity even after deletion of this element has suggested the presence of a second critical functional determinant. We have used deletion and mutational analyses to define an element, μB (TTTGGGGAA), that is essential for B-cell-specific enhancer activity in S194 myeloma cells in the absence of the octamer. Transfection analysis in a panel of lymphoid cell lines suggests that the presence of either μB or octamer leads to considerable enhancer activity in cell lines representing later stages of B-cell differentiation, whereas both elements are needed for function in cell lines representing earlier stages. Furthermore, in contrast to the results in pre-B-cell lines, both μB and octamer elements function independently in certain T-cell lines in which the μ enhancer is active.

The immunoglobulin μ heavy-chain gene is expressed at the earliest discernible pre-B-cell stage of B-cell differentiation. The enhancer associated with the μ locus (7, 14) is a critical regulatory component specifying gene activity. In fact, it has been shown in transgenic mice models that the μ enhancer is sufficient to specify the correct activation of a heterologous gene in B lymphocytes (1, 28, 41, 44). Obviously, complete characterization of the μ enhancer is likely to lead to a better understanding of the processes involved in B-cell development. The functional immunoglobulin heavy-chain gene is created by two distinct rearrangement events, and it has been suggested that transcription of the rearranging loci is a prerequisite for the rearrangement process (2, 57). This suggests that the μ enhancer may be activated at an even earlier stage of differentiation than that defined by a classical pre-B cell. Indeed, limited transfection experiments with cell lines that are in the process of undergoing DJ_H rearrangements (35) or V_H-to-DJ_H rearrangements suggest that the μ enhancer is active in these earlier cell types. Furthermore, two lines of evidence suggest that μ enhancer is active in at least some T cells. First, so-called sterile μ transcripts have been detected in certain T-cell lines (3, 23) which have been shown to initiate within the enhancer (31); second, significant expression of an introduced μ heavy-chain gene has been seen in the thymus of transgenic mice (9, 20, 51). This suggests that the μ enhancer may be activated close to the point of divergence between B and T cells during lymphocyte differentiation.

Recently in many different systems it has been shown that the activity of enhancers depends on the interaction of sequence-specific DNA-binding proteins. For the murine μ heavy-chain gene enhancer, at least five different proteins that bind to different sequence elements have been detected (6, 38, 39, 40, 43, 46, 54). Most of these factors, i.e., NF-μE1, NF-μE2, NF-μE3, and μEBP-E, can be detected in extracts derived from lymphoid and nonlymphoid cells and have therefore not been considered to be the determinants of B-cell specificity. That they play a role in enhancer activity has been clearly demonstrated by site-specific mutational analysis (24, 30, 37, 53). Kiledjian et al. (24) have further shown that multimerizing regions containing binding sites of some of these ubiquitous proteins to a high copy number leads to significant transcription activation of a test gene in a nonlymphoid cell line that is consistent with the tissue distribution of the factor itself.

The element believed to be responsible for B-cell specificity of the enhancer is an octanucleotide sequence (ATTT GCAT) which is also found in the promoters of all immunoglobulin variable region genes. This sequence element binds two factors, NF-A1 (47, 48) and NF-A2 (27, 50), of which the former is present in all cells whereas the latter is present in B cells only. (The octamer-binding factors have also been called OTF-1 and OTF-2 [12, 42], OBP-100 [52], and NF-3 [36].) However, several studies have indicated that an element other than the octamer may contribute to the B-cell specificity of this enhancer. For example, mutations of the octanucleotide in the 300-base-pair (bp) *Pvu*II-*Eco*RI fragment of the μ enhancer resulted in 10-fold-lower enhancer activity; however, in the context of the 700-bp (*Xba*I-*Eco*RI) μ enhancer, hardly any loss of activity was seen (30). Similarly, the μ enhancer A domain (*Xba*I-*Dde*I) defined by Kiledjian et al. (24) retains 40 to 50% of wild-type activity and does not include the octamer sequence. Even in the earliest reports identifying the μ enhancer, it was proposed that a sequence not containing the octanucleotide motif contained the minimal enhancer region (14). Furthermore, it has recently been shown that a stably transfected human immunoglobulin γ heavy-chain gene in a fibroblast may be activated by a nuclear factor that does not bind to the octanucleotide sequence (5). These observations have suggested the presence of a second B-cell-specific determinant in the μ enhancer.

In this study, we have used deletion analysis and point mutagenesis to define an element, μB, that is essential for the B-cell-specific activity of domain A (24) of the μ enhancer. Enhancers mutated at either μB or octamer motifs were used to determine the relative contribution of each element to enhancer function in cell lines corresponding to

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different stages of B-cell development. Our results suggest that the presence of either element leads to considerable enhancer activity in cell lines representing later stages of B cell differentiation, whereas both elements are needed for efficient function in the two pre-B-cell lines examined. Furthermore, we have examined the activity of the μ enhancer and its mutants in T-cell lines because the presence of sterile μ transcripts in some T cells had suggested that at least one of the transcriptional determinants of this locus was functional in these cells. Our results indicate that both μB and octamer motifs function in cell lines that contain sterile μ transcripts.

MATERIALS AND METHODS

Plasmids. Immunoglobulin heavy-chain enhancer fragments described below were treated with the Klenow fragment of DNA polymerase (Boehringer Mannheim Biochemicals) to create blunt ends and then were cloned into the vector pSPCAT (35) cut with *Sma*I. (All μ enhancer numbering is from Ephrussi et al. [11].) The fragments were as follows: μE, *Hin*fI (345) to *Hin*fI (566); μE2E3, *Pst*I (380) to *Dde*I (518); μ3, *Pst*I (380) to *Bam*HI (477); and μ1, *Pst*I (380) to *Bam*HI (435). Fragments μ3 and μ1 were derived from plasmids M3 and M1, respectively (21), in which unique *Bam*HI sites were introduced to disrupt the third or first core site of the μ enhancer (Fig. 1A). The *Pst*I-*Eco*RI fragments of the μ enhancer were first subcloned into pSP64 to yield pμPR(B3) and pμPR(B1), followed by excision of the *Hin*dIII-*Bam*HI fragments. This resulted in the addition of an extra 12 bp of polylinker sequence at the *Hin*dIII end. The other fragments were as follows: μ1T, *Pst*I (380) to *Sma*I (419) [plasmid pμPR(B1) was mutagenized to create a unique *Sma*I site (M102; see below), followed by isolation of the *Hin*dIII-*Sma*I fragment]; μE(B1), *Hin*fI (345) to *Hin*fI (566) with the first core sequence mutated to a *Bam*HI site; μ0, *Hin*fI (345) to *Hin*fI (566) with mutated core 1 as well as mutation M102 (see below); μB, *Hin*fI (345) to *Dde*I (518) with mutated core 1; and μ123, *Hin*fI (345) to *Dde*I (518) with mutated core 1 as well as mutation M102.

$(\mu B)_3$-containing plasmids. Two complementary oligonucleotides carrying the μB sequence

5′-GATCGGCAAGGCTATTTGGGGAAGG-3′
5′-GATCCCTTCCCCAAATAGCCTTGCC-3′

were annealed and cloned into pTZ19U cut with *Bam*HI. A plasmid containing three copies of the μB sequence, two head to head and the third head to tail [pTZ(μB_3)], was used for further cloning into expression vectors.

$(\mu B)_3$SPCAT was constructed by excising the μB trimer from pTZ$(\mu B)_3$ with *Sma*I and *Hin*cII and cloning into pSPCAT cut with *Sma*I. The orientation of the trimer obtained from sequencing the resulting plasmid was such that the head-to-tail pair was closest to the promoter. $(\mu B)_3\Delta$56fosCAT was constructed by converting the *Sma*I site in pTZ$(\mu B)_3$ to *Xho*I by using synthetic linkers and cloning the resulting *Sal*I-*Xho*I fragment into Δ56fosCAT (15) cut with *Sal*I. $(\mu B)_3\Delta$56fosCAT contained the trimer in the orientation opposite that of $(\mu B)_3$SPCAT, that is, with the head-to-head pair nearest the promoter.

Orientations of the inserts in pSPCAT were deduced by dideoxynucleotide sequencing of all plasmids. The orientations of the enhancer fragments in plasmids pμE(B1), pμ0, pμB, and pμ123 were the same, such that the μE1 motif was distal to the simian virus 40 (SV40) promoter. All plasmids were purified by two sequential bandings through cesium chloride gradients and quantified spectrophotometrically. The conformational state of all plasmids was the same as that judged by agarose gel electrophoresis.

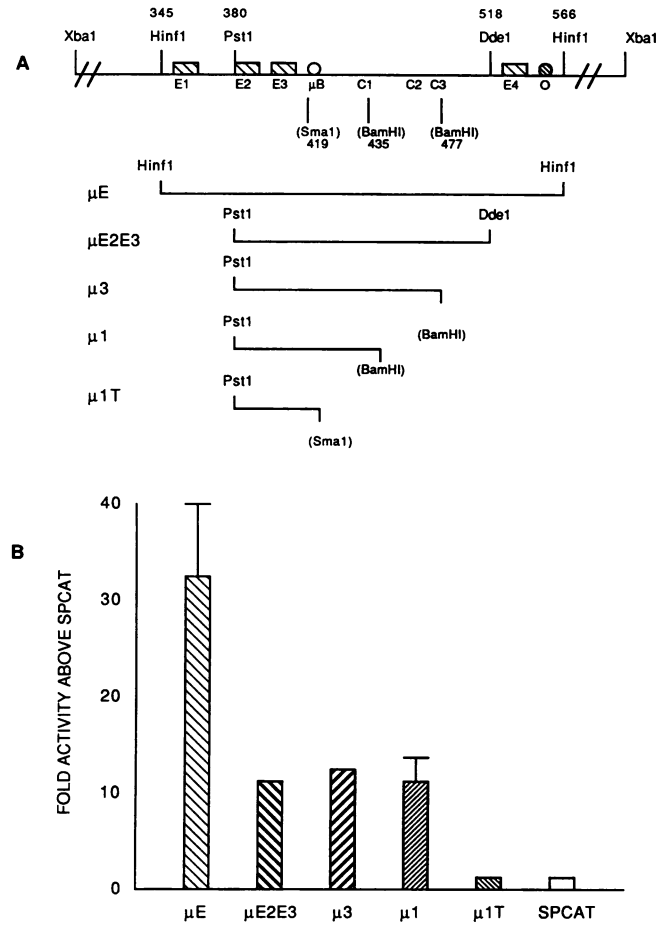

FIG. 1. Deletion analysis to map the minimal B-cell-specific domain of the immunoglobulin heavy-chain gene enhancer. (A) Enhancer fragments used to define the minimal activating domain. The top line shows a schematic representation of the μ enhancer (nucleotide numbering is from Ephrussi et al. [11]). The four homologous motifs E1 to E4 are indicated as boxes. These sequences bind the ubiquitous proteins NF-μE1, NF-μE2, and NF-μE3. Three regions that are homologous to the SV40 enhancer core sequence are indicated as C1 to C3. The octamer motif that interacts with B-cell-specific and ubiquitous proteins NF-A2 and NF-A1 is shown as the shaded circle (labeled O). Restriction enzyme sites shown in parentheses below the line represent different site-specific mutations that were used to make μ enhancer deletions (indicated as μE2E3, μ3, μ1, and μ1T) used in this study. (B) Activities of the μ enhancer subfragments in murine S194 myeloma cells. The DNA segments described above were cloned into the vector pSPCAT and transfected into S194 cells by the DEAE-dextran procedure. Levels of chloramphenicol acetyltransferase (CAT) enzyme activity were assayed in extracts made 48 h after transfection and are indicated on the *y* axis as the activity of the test plasmid above pSPCAT activity. Assays were conducted with 100 μg of protein for 1 h. Transfections were done at least three independent times in duplicate, and the standard deviations for the experiments are indicated by error bars. No error bar indicates a standard deviation of less than 1%.

Mutagenesis. Site-directed mutagenesis was carried out by using the gapped duplex methodology (25) for mutants M100, M102, M104, M105, and M106. Mutant M103 was generated using the in vitro mutagenesis system kit, version

2 (Amersham Corp.). Mutant M101 was generated by modification of a polymerase chain reaction protocol described by Yon and Fried (58). Oligonucleotides used for mutagenesis were as follows (mutated residues are underlined): M100, CCCCAAATAGTACTGCCACATG; M101, CCCC AAATATTCTTGCCACATG; M102, CCCTTCCCCGGG TAGCCTTGCC; M103, CCATTTTCCCTTCTTTAAATAG CC; M104, CCATTTTCCCGGGCCCAAATAGCC; M105, GGATCCATTTTAAATTCCCCAAATAGCC; and M106, GTGGATCCATGGGCCCTTCCCC. The various mutations were analyzed by cloning the *Hin*fI (345)-*Dde*I (518) fragments of the mutated enhancers into pSPCAT, followed by transfection into S194 cells. Orientations of all enhancer fragments were the same, and the mutant sequences were verified by dideoxynucleotide sequencing.

Cell lines. The following cell lines representing B-cell development stages, T-cell lines, and nonlymphoid cells were used for transfection analysis. 300-19 is an Abelson virus-transformed pre-B-cell line that undergoes V_H-to-DJ_H rearrangements in culture (4). PD31 is an Abelson virus-transformed pre-B-cell line that undergoes κ light-chain rearrangement in culture (32). M12.A2 is an I^+E^+ murine B-cell line (18). DHL-9 is a human B-cell line (56). S194 is a murine myeloma cell line and represents the final differentiation stage of the B cell. T-cell lines used which have sterile μ transcript are EL4 and RL male 11 (3). BW5147 is a T cell lacking sterile μ transcript (23). HeLa (a human cervical carcinoma cell line) and NIH 3T3 (a murine fibroblast cell line) were used as the nonlymphoid controls.

PD31, M12.A2, EL4, and RL male 11 were grown in RPMI 1640 medium supplemented with 5% inactivated fetal calf serum (GIBCO Laboratories), 0.0004% β-mercaptoethanol, penicillin, and streptomycin. DHL-9 cells were grown identically except with 10% inactivated fetal calf serum. BW5147 and S194 were grown in Dulbecco modified Eagle medium supplemented with 5% inactivated fetal calf serum and 5% calf serum, with the β-mercaptoethanol, penicillin, and streptomycin conditions described above. HeLa cells were grown in Dulbecco modified Eagle medium containing 10% donor calf serum, penicillin, and streptomycin.

Transfections and CAT enzyme analysis. Transient transfections of all cell lines except NIH 3T3 were carried out by using the DEAE-dextran protocol (19). NIH 3T3 cells were transfected by the calcium phosphate method (17). DHL-9 cells were transfected with modifications described by D. Loh (personal communication). Typically, 1×10^6 to 2×10^6 adherent cells (M12.A2, HeLa, and NIH 3T3) or 10^7 nonadherent cells (all others) were transfected with 2 μg or 5 to 10 μg of supercoiled plasmid DNA, respectively. All transfections were repeated at least twice in duplicate. Cellular extracts were prepared 44 to 48 h after transfection, and CAT enzyme levels were assayed as previously described (16, 35). The conditions of the assays varied among cell lines as follows: S194, 100 μg of extract for 1 h; M12.A2, 200 μg for 5 h; DHL-9, 200 μg for 2 h; PD31, 300-19, EL4, RL male 11, and BW5147, 300 μg for 5 h; and HeLa and NIH 3T3, 200 μg for 3 h.

DNase I footprinting. DNase I footprinting was carried out essentially as described by Lee et al. (29). The μ enhancer-containing pSPCAT-derived plasmids were cut at the *Eco*RI site, treated with calf intestinal phosphatase, and end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. After further digestion with *Hin*dIII, the μE fragment was purified on 12% polyacrylamide gels. In a typical footprinting reaction, 30,000 cpm of radioactive DNA was incubated with 150 μg of crude nuclear extracts (10) for 30 min at 25°C. Mg^{2+} and Ca^{2+} were added to final concentrations of 5 and 2.5 mM, respectively, followed by DNase I (final concentrations, 0.25 μg/ml for free DNA and 5 μg/ml for DNA and extract). After 1 min, the reaction was quenched with 10 mM EDTA–0.5% sodium dodecyl sulfate–0.1 M NaCl–125 μg of tRNA per ml. After extraction with phenol and chloroform, the reactions were analyzed by electrophoresis through 8% polyacrylamide gels containing 8 M urea.

RESULTS

Definition of a minimum B-cell-specific μ enhncer sequence. The μ enhancer and subfragments (Fig. 1A) thereof were tested after cloning into the previously characterized vector pSPCAT. This plasmid carries the bacterial CAT gene (35) transcribed from the complete SV40 early promoter lacking the viral enhancer. Plasmids were transiently transfected into S194 (murine myeloma) cells by the DEAE-dextran procedure, followed by analysis of CAT enzyme activity 44 to 48 h after transfection. A 220-bp *Hin*fI fragment was used as the wild-type enhancer because an earlier report has ascribed full activity to this fragment when compared with the 700-bp *Xba*I-*Eco*RI fragment originally identified (13). The *Hin*fI fragment contains the previously identified nuclear factor-binding sites μE1, μE2, μE3, and μE4 and the octamer sequence (Fig. 1A) but does not contain site E identified more recently by Peterson et al. (39). To define a minimal B-cell-specific μ enhancer domain, large deletions of this 220-bp fragment were tested by transient transfection into S194 cells. Consistent with an earlier report (14), the 140-bp *Pst*I-*Dde*I fragment was approximately 30% as active as the full μ enhancer (Fig. 1). Removal of an additional 40 bp (fragment μ3) or even 80 bp (fragment μ1) did not decrease the enhancer activity significantly (Fig. 1). The 62-bp μ1 fragment contains the previously identified sites μE2 and μE3 and an additional 20 bp 3′ to the μE3 site. To determine whether the activity of this fragment was due only to μE2 and μE3, a unique *Sma*I restriction enzyme site was introduced 3′ to μE3 by site-specific mutagenesis, and a deletion mutant extending to this site (μ1T; Fig. 1A) was checked for enhancer activity. This enhancer deletion was unable to enhance transcription in S194 cells, showing that maintaining μE2 and μE3 intact was not sufficient to provide enhancer activity (Fig. 1B), suggesting the presence of a critical determinant 3′ to μE3. We will refer to the element 3′ of μE3 as μB.

To more precisely define μB, clustered point mutations were generated throughout the 20-bp sequence. Two or three base pairs at a time were changed between nucleotides 410 and 430 (numbering system of Ephrussi et al. [11]), and the effects of the mutations on the enhancer activity of domain A (*Hin*fI-*Dde*I) of the μ enhancer were analyzed by transfection into S194 plasma cells. The results are summarized in Fig. 2. Alteration of the first 3 bp did not affect enhancer activity at all. Mutations of residues 412 to 414, 425 to 427, or 428 to 430 (M101, M105, or M106, respectively) decreased activity to approximately 50 to 60% of the wild-type levels. Mutants M102 and M104 had the most detrimental effect on enhancer function and decreased activity to background levels and to 7% of the wild-type activity, respectively. Surprisingly, mutant M103, which falls in between, did not affect enhancer function. Although this might suggest the presence of two possible elements, we favor the interpretation that the core of the μB determinant covers bp 416 to 424 (TTTGGGGAA), with some flexibility in the internal residues. This view is supported by DNase I footprinting anal-

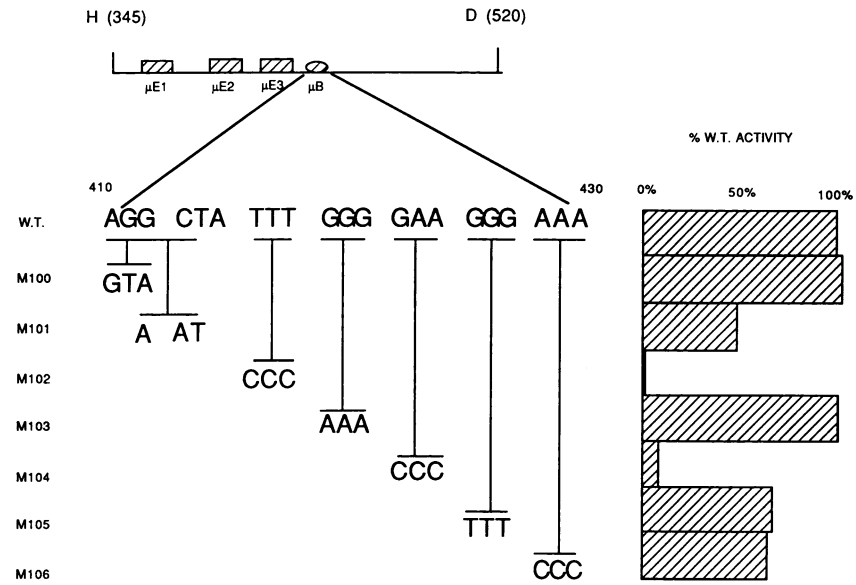

FIG. 2. Localization of the μB element by analysis of enhancer mutations. Clustered mutations were generated in the 20 bp 3′ from the μE3 sequence as described in Materials and Methods. The wild-type (W.T.) sequence and the alterations made are as indicated (base pair numbering is from Ephrussi et al. [11]). Mutations were analyzed after cloning the *Hin*fI-*Dde*I fragment of the enhancer into pSPCAT (35) and transfection of the appropriate plasmids into S194 cells. The activity of each mutant is expressed as a percentage of the activity obtained with the unmutated *Hin*fI-*Dde*I enhancer fragment. Values are results of two transfections performed in duplicate with less than 10% standard deviation.

ysis of wild-type and mutant enhancer sequences, which identified a factor interacting with both wild-type and M103 sequences but not with M102 and M104 mutations that abolish μB activity (discussed below).

To check whether the μB motif alone could activate B-cell-specific transcription, we tested a plasmid containing a trimer of the μB element located upstream from the SV40 early promoter in pSPCAT (see Materials and Methods). Transfection into S194 plasma cells showed no detectable activity above that of the enhancerless vector. Because the SV40 early promoter contains multiple Sp1-binding sites, we sought to confirm these results with a CAT plasmid containing a minimal promoter. For this, the trimer of the μB site was inserted into the unique *Sal*I site located 56 bp upstream of a truncated c-*fos* gene promoter (15). However, even in this context the μB sites were unable to confer increased transcriptional activity (data not shown). Apparently, this sequence element alone is not sufficient to act as a transcriptional activator, although we cannot unequivocally rule out the possibility that μB has stringent spacing requirements that are not met in our constructs.

Contribution of μB or octamer elements to enhancer activity in S194 cells. Identification of a second critical element, in addition to the octamer, raises a question about the utilization of each motif for enhancer function. To determine the relative contributions of μB or octamer to the activity of the full μ enhancer, pSPCAT-derived plasmids containing the enhancers depicted in Fig. 3B were transfected and analyzed for expression in S194 plasma cells. Because mutational analysis has not yet shown any contribution of μE4 to enhancer function, we have interpreted results with the smaller *Hin*fI-*Dde*I fragments as being primarily due to the absence of the octanucleotide sequence. pμE(B1) contains the complete enhancer, retaining both octamer and μB elements (B1 denotes mutations in the first core sequence that has been shown not to affect enhancer function in plasma cells [21]), and activates expression approximately 30-fold in S194 cells. Plasmid pμB contains an enhancer fragment terminating at the *Dde*I site which retains the μB element but not the octamer sequence. Absence of the octamer sequence in this fragment reduces enhancer activity to 50% of that of the full enhancer. Plasmid pμ0 contains the complete enhancer with a mutated μB element (mutation M102 in Fig. 2) and is only 36% as active as pμE(B1). Lastly, if octamer and μB elements were both missing (pμ123), no activity above the background contributed by the enhancerless plasmid pSPCAT was seen (Fig. 4). This analysis shows that although mutation of the μB motif has a greater effect on enhancer function, a fragment containing either μB or octamer retains significant enhancer activity in S194 cells and that the effect of both being present together is additive.

Transfection analysis in nonlymphoid cell lines. To confirm that the negative effects of μB mutations were restricted to lymphoid cells only, we analyzed the activities of both deletion mutants (Fig. 1A) and point mutants (Fig. 3B) of the μ enhancer in nonlymphoid cells. In HeLa cells, deletions μE2E3 and μ1 (containing μE2, μE3, and μB motifs) were somewhat more active than the full enhancer (Fig. 5A). Further removal of approximately 20 bp containing the μB region (μ1T) caused a further increase of enhancer activity in this cell type (Fig. 5A), in contrast to the drastic reduction of activity seen in S194 B cells (Fig. 1B). In addition, murine NIH 3T3 cells were transfected with plasmid pμE, pμ0, pμB, or pμ123. Although the full enhancer (in pμE) conferred no activity above that of the enhancerless vector pSPCAT, removal of the octamer (in pμB), the μB element (in pμ0), or both elements (in pμ123) resulted in mutant enhancers that showed slightly increased activity (Fig. 5B). Taken together, these observations suggest that the μB sequence does not function as a positive enhancer element in nonlymphoid cells. We have not further investigated the basis of the somewhat greater activity of the mutant enhancers.

Contribution of μB and octamer elements to enhancer

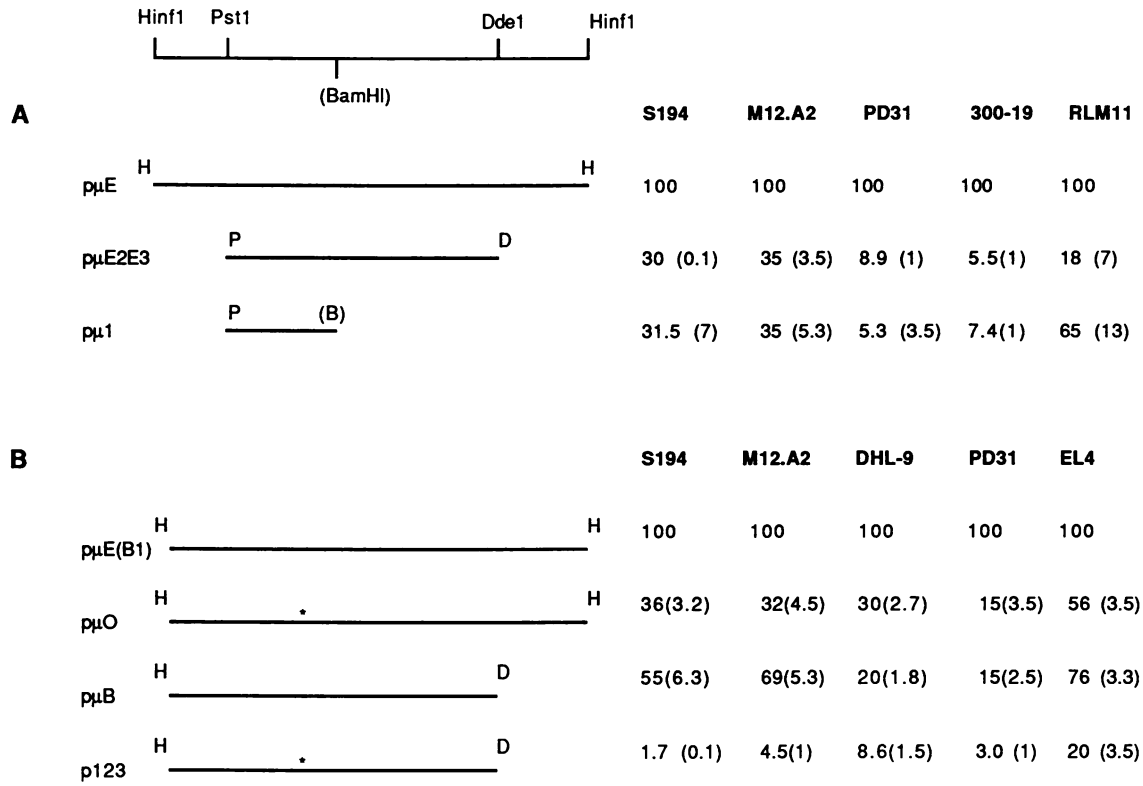

FIG. 3. Summary of transfection analysis in B- and T-cell lines. The *Hin*fI fragment of the μ enhancer (A) is shown with relevant restriction sites. The *Bam*HI site indicated was introduced by site-specific mutagenesis at the first core sequence, C1 (see Fig. 1A). Asterisks in pμ0 and pμ123 (B) indicate mutation M102 in the μB element. All enhancer fragments in panel B also contain the *Bam*HI mutation in C1, which has been shown not to affect enhancer activity. The activity of each enhancer is presented as a fraction of the activity of the full enhancer, which is considered to be 100% after subtracting the background contributed by the enhancerless vector pSPCAT (see text for absolute values). Numbers in parentheses show the standard deviations observed among at least four experiments. Abbreviations: H, *Hin*fI; P, *Pst*I; D, *Dde*I; B, *Bam*HI. Cell lines: S194, murine plasma cells; M12.A2, murine B cells; DHL-9, human B cells; PD31 and 300-19, Abelson virus-transformed murine pre-B cells; EL4 and RLM11, murine T cells.

activity during B-cell differentiation. To date, evaluation of the contribution of μ enhancer motifs to general or cell-specific transcription has been limited to analysis in fibroblast and plasma cell lines. Because plasma cells represent only the most terminal stage of B-cell differentiation, these analyses, though useful in identifying important sequences, have not provided insights into the possible role of the various motifs for enhancer function during B-cell differentiation. For example, one of these elements may be activated at an earlier developmental stage than the other. We have used cell lines representing different stages of B-cell differentiation (22) to investigate the contribution of μB and octamer sequences to enhancer activity in these cell types. Comparative analysis of enhancer activities between cell lines is complicated primarily by the differences in transfection efficiencies. To ensure the best possible comparisons, we have expressed the activity of the enhancer deletions or mutations as a fraction of the activity of the full enhancer in each cell line. Moreover, transfection titrations were carried out in each cell type by varying the amount of DNA used to ensure that the experiments were performed in the linear response range for the amount of DNA transfected (data not shown).

In the murine IA-expressing B-cell line M12.A2 (18), the full μ enhancer activates transcription from the SV40 promoter by approximately eightfold. Similar to the results in S194 plasma cells, both pμ0 (32% of μ enhancer) and pμB (69% of μ enhancer) were quite active in M12 cells (Fig. 3B). As a second cell line representing the B-cell stage of development, we used the human mature B-cell line DHL-9 (56), which expresses surface immunoglobulin. The complete μ enhancer stimulates transcription approximately 12-fold more than the SV40 promoter in this cell line. However, the activities of pμ0 and pμB were about 30 and 20%, respectively, that of the plasmid containing the full μ enhancer (Fig. 3B). Finally, as an example of an even earlier stage of B-cell differentiation, we chose the murine pre-B-cell line PD31 (32). In these cells, the full enhancer conferred approximately eightfold transcriptional enhancement, and removal of either the octamer or the μB motif caused a significant decrease in enhancer function [pμ0 and pμB were only 15% as active as pμE(B1); Fig. 3B]. Consistent with these observations, an enhancer fragment not containing the octamer was inactive in a second pre-B-cell line, 300-19 (Fig. 3A). In all B-lymphoid cell lines used, plasmid pμ123 containing an enhancer fragment lacking both the octamer and μB motifs had activity comparable to that of the enhancerless vector pSPCAT (Fig. 3B). These results suggest that the two critical determinants of enhancer activity do not act at different stages of B-cell development. Rather, early in development, the μB element functions inefficiently in the absence of the octamer, whereas at later stages it alone can confer significantly transcriptional activation. Enhancer activity dependent only on the octamer sequence remains roughly equivalent at all differentiation stages. In this model, DHL-9 cells

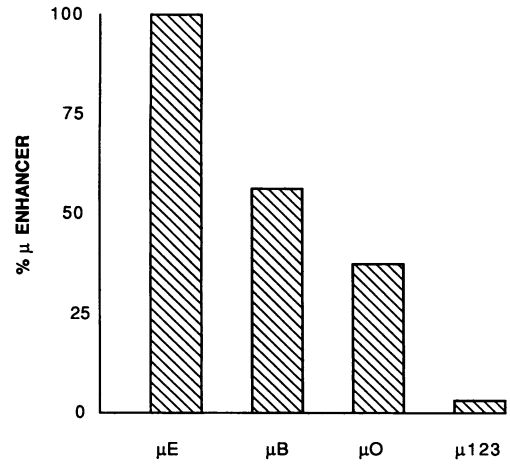

FIG. 4. Contribution of μB and octamer elements to enhancer activity in the plasma cell line S194. Enhancer derivatives shown in Fig. 3 containing both the octamer and μB elements [pμE(B1)], the octamer alone (pμ0), the μB element alone (pμB), or neither element (pμ123) were assessed for enhancer activity by transfection into the plasma cell line S194. All enhancer fragments were present in the same orientation with respect to the promoter (such that the E1 element was distal to the promoter); the activity of each is expressed as a percentage of the full μ enhancer activity. Transcriptional enhancement conferred by the presence of the enhancer [pμE(B1)CAT compared with pSPCAT)] was approximately 30-fold. Extracts were made 48 h after transfection and assayed for CAT activity as described in Materials and Methods. Activities of the enhancer mutations, expressed as percentages of the activity of the full enhancer, are indicated on the *y* axis; the results shown represent at least two independent experiments performed in duplicate (see Fig. 3 for standard deviations).

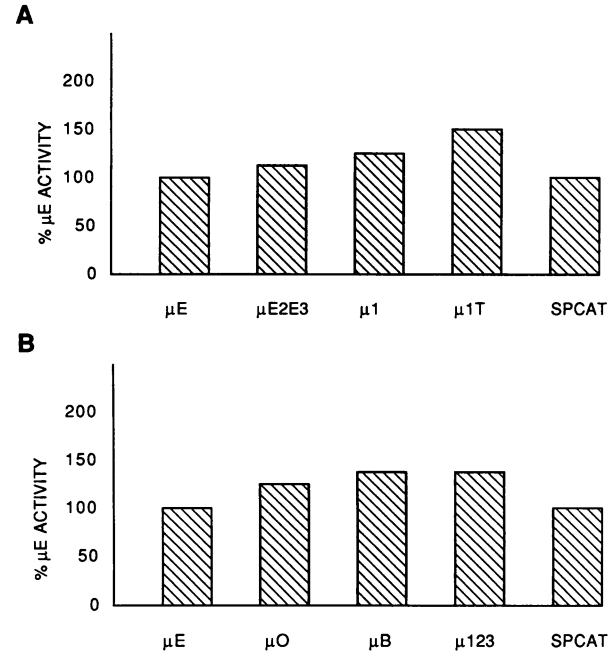

FIG. 5. Transfection analysis of μ enhancer derivatives in nonlymphoid cell lines. (A) Activities of the μ enhancer subfragments in HeLa cells. Large-deletion mutants of the μ enhancer shown in Fig. 1A were transfected into to HeLa cells by the DEAE-dextran procedure (see Materials and Methods). After 48 h, cells were harvested and levels of CAT enzyme were assayed, using 300 μg of protein in a 2-h incubation. Levels of activity are represented as percentages of the full μ enhancer activity. Results represent at least two independent transfections with standard deviations of less than 20%. Plasmid pSV2CAT, containing the SV40 enhancer, showed an approximately 40-fold increase in CAT activity over the enhancerless construct pSPCAT in the same experiments. (B) Analysis of μ enhancer mutations in NIH 3T3 cells. Plasmids containing μ enhancer derivatives (depicted in Fig. 3B) that lack both octamer and μB elements (pμ123CAT), contain only one of the two elements (pμBCAT and pμ0CAT), or retain both elements (pμECAT) were transfected into the murine fibroblast line NIH 3T3 by the calcium phosphate method (see Materials and Methods). The activity of each plasmid is expressed as a percentage of the full enhancer activity. Results shown represent at least three independent experiments done in duplicate with a standard deviation of less than 20%. Plasmid J21MO (55) containing the Moloney murine leukemia virus enhancer gave a 17-fold increase in activity over background in the same set of experiments.

represent a stage that is intermediate between those represented by PD31 and M12.A2 cells.

μ enhancer activity in T-cell lines. Partial immunoglobulin heavy-chain gene rearrangements in T-cell lines (3, 8, 26), presence of sterile μ transcripts (3), and the expression of exogenous μ heavy-chain gene in the spleen as well as the thymus of transgenic mice (9, 20) has suggested that T cells are permissive for at least one of the regulatory regions associated with the μ locus. We have chosen three T-cell lines that have been previously characterized with respect to the presence or absence of sterile μ transcripts for transfection analysis of the μ enhancer.

In EL4 and RL male 11 cells that contain sterile μ transcripts, the full μ enhancer was active (Fig. 3). However, in a T-cell line lacking sterile μ transcripts, BW5147, the μ enhancer was inactive (data not shown; transfection of plasmid pSV2CAT that contains the SV40 enhancer showed 20-fold activity above background, indicating that BW5147 cells were transfectable). To determine whether the observed μ enhancer activity in EL4 and RL male 11 lines was dependent on either the octamer or μB element, transfection experiments were done by using the mutated enhancers described above. In EL4 cells (Fig. 3B), removal of either the octamer sequence or the μB sequence from the enhancer (plasmid pμB or pμ0, respectively) showed at most a two-fold decrease in enhancer activity, suggesting that each element could function independently. Similarly, the small μ1 fragment of the μ enhancer lacking the octamer site retained considerable activity (Fig. 3A) in RL male 11 cells. (The reason for the anomalously low activity of the larger μE2E3 fragment in this cell line is unclear at present.) This pattern of activity in EL4 and RL male 11 cells was similar to that observed in cell lines representing later stages of B-cell differentiation (S194 and M12.A2). However, in contrast to the results in B-cell lines, the enhancer without both μB and octamer motifs still retained significant activity in both of these T-cell lines. This observation suggests the presence of other, possibly T-cell-specific, determinants of enhancer activity and reemphasizes the modular and complex nature of the μ enhancer.

DNase I footprint analysis of the minimal μ enhancer. To search for a *trans*-acting factor interacting with the μB sequence element, we carried out in vitro DNase I footprint analysis, using nuclear extracts derived from lymphoid and nonlymphoid cell lines and a radioactive DNA probe derived from the wild-type and mutant enhancers. Unfortunately, only a few bands were generated by DNase I treatment in the region of the μB sequence; however, there was clear protection of a broad band in A20 (murine B) cell extracts (in the region bracketed as μB) compared with the free fragment

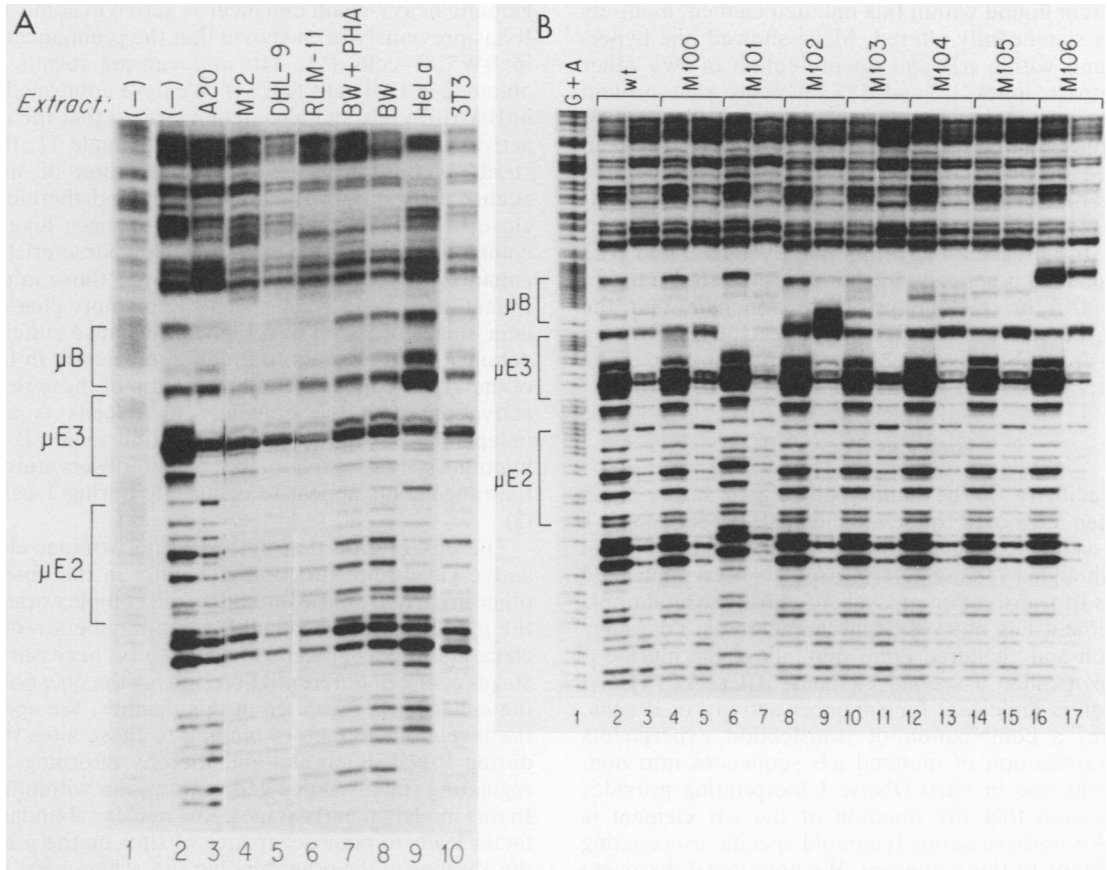

FIG. 6. DNase footprint analysis of the μB element. (A) Cell type specificity of a *trans*-acting factor interacting with the μB locus. DNase I footprinting was performed on the 170-bp *Hin*fI-*Dde*I μE fragment (μ170) obtained from pμBCAT. Radioactive labeling of the fragment and the footprinting assay were done as described in Materials and Methods. Assays were performed in the absence of nuclear extract (lane 2) or in the presence of 150 μg of crude nuclear extract derived from murine B-cell lines A20 and M12.A2 (lanes 3 and 4), human B-cell line DHL-9 (lane 5), murine sterile μ^+ T-cell line RL male 11 (lane 6), murine sterile μ^- T-cell line BW5147 (lane 8) or BW5147 activated with phytohemagglutinin (lane 7), human cervical carcinoma cell line HeLa (lane 9), or the murine fibroblast cell line NIH 3T3 (lane 10). A G+A ladder of the same fragment was run as a marker to identify enhancer element locations (lane 1). (B) DNase I protection analysis of wild-type (wt) and mutant μB sequences. SPCAT plasmids described in Fig. 2 were used to obtain radioactively labeled μ170 fragments (see Materials and Methods) containing wild-type or clustered mutant μB sequences (M100 to M106). Footprinting assays were done as previously described in the absence (lanes 2, 4, 6, 8, 10, 12, 14, and 16) or presence (lanes 3, 5, 7, 9, 11, 13, 15, and 17) of 150 μg of murine B-cell nuclear extract. A G+A ladder of the wild type (lane 1) identifies the location of ubiquitous motifs μE2 and μE3 as well as the μB region. Mutants M102 and M104 affect the functional activity of the μB element.

(Fig. 6A, lanes 2 and 3). In addition, protection of the previously identified μE3 and μE2 elements was also clearly evident. These results suggest the identification of a factor that interacts with the μB element.

To confirm this interpretation and further study the distribution of this factor in different cell lines, the footprint analysis was extended to include a panel of nuclear extracts (Fig. 6A, lanes 3 to 10). We observed protection against DNase cleavage within the μB domain in extracts derived from A20 (murine B) (lane 3), M12.A2 (murine B) (lane 4), DHL-9 (human B) (lane 5), and RL male 11 (murine T) (lane 6) cells but not in extracts derived from BW (murine T) cells with or without treatment with phytohemagglutinin (lanes 7 and 8), HeLa (human cervical carcinoma) cells (lane 9), or NIH 3T3 (murine fibroblast) cells (lane 10). In contrast, protection of the μE3 and μE2 domains remained approximately equivalent in all extracts, consistent with the previously proposed tissue distribution of the factors binding to these motifs. The close correlation between protection over the μB sequence and extracts derived from cells in which this element has been shown to contribute to enhancer function (M12.A2, DHL9, and RL male 11) is entirely consistent with the identification of a lymphoid-specific factor interacting with this motif.

To further correlate the in vitro binding activity with the functional analysis of the μ enhancer, we analyzed the set of mutant μB sequences by DNase I footprint analysis (Fig. 6B). If the protection observed in vitro were related to the in vivo activity, the mutations affecting enhancer function should show an altered pattern of protection compared with the wild-type sequence. Mutants 100, 101, 105, and 106 (Fig. 6B, lanes 4 and 5, 6 and 7, 14 and 15, and 16 and 17, respectively), which flank the μB element defined by transfection analysis, showed a protection pattern similar to that of the wild-type sequence (Fig. 6B, lanes 2 and 3) in A20 (murine B) cell extracts. However, mutants that reduced μB-dependent enhancer function showed marked differences in the DNase I digestion pattern within this sequence. M102 showed two hypersensitive bands within μB in the presence of B-cell extracts (compare lanes 8 and 9), showing

that if a protein bound within this mutated element at all, its binding was significantly altered. M104 showed one hypersensitive band within μB and no protection of two other bands (compare lanes 12 and 13), showing that protein binding was significantly reduced. Interestingly, M103, which is a fully functional enhancer mutation lying between M102 and M104 that affect function, showed a protection pattern like that of the wild-type sequence, indicating that the protein can bind to this mutated element (compare lanes 10 and 11). In all cases, the protection over μE3 and μE2 domains remained practically unchanged, providing a useful internal control in this analysis. We conclude that the footprinting studies identify a lymphoid-specific *trans*-acting factor that is probably responsible for in vivo function of the μB element.

DISCUSSION

B-cell specificity of the immunoglobulin μ heavy-chain gene has been attributed to the octanucleotide sequence AT TTGCAT, which interacts with the B-cell-specific protein NF-A2. Although a second determinant has been implicated on the basis of transfection analysis of enhancer mutations, such an element has not yet been characterized. We have used deletion and clustered point mutants of the murine μ enhancer to localize a second element, μB (TATTTGGG GAA), which is important for enhancer activity in B cells. Furthermore, a combination of transfection experiments involving transfection of mutated μB sequences into nonlymphoid cells and in vitro DNase I footprinting provides strong indication that the function of the μB element is mediated by a positive-acting lymphoid-specific *trans*-acting factor that binds to this sequence. We note that Libermann et al. have independently identified and examined the effects of this sequence element and reached similar conclusions (33). Previous analysis of the A domain of the μ enhancer showed that point mutations in any of the μE1, μE2, and μE3 motifs had little effect on enhancer activity (24). In contrast, point mutations in the μB element within domain A essentially abolish enhancer function.

Araki et al. (5) have demonstrated that a human immunoglobulin G gene that is stably transfected into murine fibroblasts can be activated by microinjection of nuclear factors derived from murine B cells. The major protein purified on the basis of this assay binds to a portion of the human gene that corresponds to the μB determinant identified in this study. However, it is difficult to directly compare their results with ours because the role of this protein-binding site within the gene in the observed derepression is not clear. Our experiments provide direct evidence for the existence of a determinant that is critical for enhancer function in B lymphocytes.

In addition, we have investigated the contribution of the μB element and of the octamer sequence for enhancer activity in cell lines representing different stages of B-cell development and in T cells. We show that both the μB and octamer motifs contribute to enhancer activity at all stages of B-cell development. Interestingly, in cell lines representing later stages of development (e.g., M12.A2 and S194), the μB motif particularly can function efficiently by itself, whereas in two pre-B-cell lines (PD31 and 300-19), the μB element is unable to function well in the absence of the octamer motif. In this regard, the human B-cell line DHL-9 has a phenotype closer to that of the pre-B cell lines than that of the more mature ones.

Second, we have formally demonstrated that the immunoglobulin heavy-chain enhancer is active in some T-cell lines. It has previously been shown that the μ enhancer is inactive in BW5147 cells (13, 34), and varying results have been obtained in EL4 cells (23, 34). We have confirmed the results in BW5147 cells and also demonstrated that the enhancer is active in two cell lines, EL4 and RL male 11, that contain sterile μ transcripts. Because activation of the μ locus occurs early during B-cell ontogeny and therefore perhaps close to the stage where the two lineages diverge from a common precursor, we expected that characteristics of the μ enhancer in T cells would be similar to those in pre-B cells. In fact, activity in T cells seems to be more characteristic of later stages of B-cell development because either μB or the octamer motif appears to function efficiently in the absence of the other. A possible interpretation of these results is that activation of the μ enhancer in T cells is a later and independent event from its activation in pre-B cells. This hypothesis may explain the recent observation that DJ_H rearrangements appear to occur late during T-cell ontogeny (8).

The observation that both μB and octamer elements are active yet do not function efficiently in the absence of the other in early B cells exemplifies the complex organization of the μ enhancer. Specifically, what may be a redundancy of elements in plasma cells appears to be necessary at earlier stages of B-cell differentiation, raising the question as to why the enhancer is regulated in this manner. We speculate that the levels of the factors binding to these sites may change during B-cell differentiation, thereby affording a means of regulating stage-specific gene expression within this lineage. In this model, at early stages, low nuclear abundance of each factor leads to poor occupancy of sites on the μ enhancer in the absence of the other binding site. The μ gene is activated early as a result of the presence of two functional binding sites in its enhancer compared with another gene whose expression is dependent on only one of these motifs. At later stages, the levels of both proteins are much higher, allowing the expression of other genes dependent on either motif while being reflected as a redundancy in the context of the μ enhancer. We note that this hypothesis is supported by analysis of the expression of the *oct-2* gene, which encodes the B-cell-specific octamer-binding protein NF-A2/OTF-2 (49). In the panel of cell lines examined, expression of the *oct-2* gene was much lower in two pre-B-cell lines (38B9 and 70Z/3) than in more mature lines such as WEHI 231 and BJAB.

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LITERATURE CITED

1. **Adams, J. M., A. W. Harris, C. A. Pinkert, L. M. Corcoran, W. S. Alexander, S. Cory, R. D. Palmiter, and R. L. Brinster.** 1985. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. Nature (London) **318**:533–538.
2. **Alt, F. W., T. K. Blackwell, R. A. Despinho, M. G. Reth, and G. D. Yancopoulos.** 1986. Regulation of genome rearrangement

events during lymphocyte differentiation. Immunol. Rev. **89:** 5–30.
3. **Alt, F. W., N. Rosenberg, V. Enea, E. Siden, and D. Baltimore.** 1982. Multiple immunoglobulin heavy-chain gene transcripts in Abelson murine leukemia virus-transformed lymphoid cell lines. Mol. Cell. Biol. **2:**386–400.
4. **Alt, F. W., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore.** 1981. Organization and reorganization of immunoglobulin genes in A-MuLV-transformed cells: rearrangement of heavy but not light chain genes. Cell **27:**381–390.
5. **Araki, K., H. Maeda, J. Wang, P. Kitamura, and T. Watanabe.** 1988. Purification of a nuclear trans-acting factor involved in the regulated transcription of a human immunoglobulin heavy chain gene. Cell **53:**723–730.
6. **Augereau, P., and P. Chambon.** 1986. The mouse immunoglobulin heavy-chain enhancer: effect on transcription *in vitro* and binding of proteins present in HeLa and lymphoid B cell extracts. EMBO J. **5:**1791–1797.
7. **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.
8. **Born, W., J. White, J. Kappler, and P. Marrack.** 1988. Rearrangement of IgH genes in normal thymocyte development. J. Immunol. **140:**3228–3232.
9. **Brombacher, F., M. C. Lamers, G. Köhler, and H. E. Eibel.** 1989. Elimination of CD8+ thymocytes in transgenic mice expressing an anti-Ly+2.2 immunoglobulin heavy chain gene. EMBO J. **8:**3719–3726.
10. **Dignam, J. D., R. M. LeBowitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
11. **Ephrussi, A., G. M. Church, S. Tonegawa, and W. Gilbert.** 1985. B lineage-specific interactions of an immunoglobulin enhancer with factors *in vitro*. Science **227:**134–140.
12. **Fletcher, C., N. Heintz, and R. G. Roeder.** 1989. Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2b gene. Cell **51:**773–781.
13. **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.
14. **Gillies, S. D., S. Morrison, V. 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.
15. **Gilman, M. Z., R. N. Wilson, and R. A. Weinberg.** 1986. Multiple protein-binding sites in the 5′-flanking region regulate c-*fos* expression. Mol. Cell. Biol. **6:**4305–4316.
16. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
17. **Graham, F. L., and A. J. Van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52:**456–467.
18. **Griffith, I. J., N. Nabairi, S. Ghogawala, C. G. Chase, M. Rodriguez, D. J. McKean, and L. H. Glimcher.** 1988. Structural mutation affecting intracellular transport and cell surface expression of murine class II molecules. J. Exp. Med. **167:** 541–555.
19. **Grosschedl, R., and D. Baltimore.** 1985. Cell type specificity of immunoglobulin gene expression is regulated by at least three DNA sequence elements. Cell **41:**885–897.
20. **Grosschedl, R., D. Weaver, D. Baltimore, and F. Constantini.** 1984. Introduction of a μ immunoglobulin gene into the mouse germ line: specific expression in lymphoid cells and synthesis of functional antibody. Cell **38:**647–658.
21. **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.
22. **Kelley, D. E., B. A. Pollok, M. L. Atchison, and R. P. Perry.** 1988. The coupling between enhancer activity and hypomethylation of κ immunoglobulin genes is developmentally regulated. Mol. Cell. Biol. **8:**930–937.
23. **Kemp, D. J., A. W. Harris, S. Corey, and J. M. Adams.** 1980. Expression of the immunoglobulin Cμ gene in mouse T and B lymphoid and myeloid cell lines. Proc. Natl. Acad. Sci. USA **77:**2876–2880.
24. **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.
25. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
26. **Kurosawa, Y., H. VonBoehmer, W. Haas, H. Sakano, A. Traunecker, and S. Tonegawa.** 1981. Identification of D segments of immunoglobulin heavy chain genes and their rearrangement in T lymphocytes. Nature (London) **290:**565–570.
27. **Landolfi, N. F., J. D. Capra, and P. W. Tucker.** 1986. Interaction of cell-type-specific nuclear proteins with immunoglobulin V_H promoter region sequences. Nature (London) **323:**548–551.
28. **Langdon, W. Y., A. W. Harris, S. Cory, and J. Adams.** 1986. The c-myc oncogene perturbs B lymphocyte development in Eμ-myc transgenic mice. Cell **47:**11–18.
29. **Lee, W., P. Mitchell, and R. Tjian.** 1987. Purified transcription factor AP 1 interacts with TPA-inducible enhancer elements. Cell **49:**741–752.
30. **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.
31. **Lennon, G. G., and R. P. Perry.** 1985. Cμ containing transcripts initiate heterogeneously within the IgH enhancer region and a novel 5′-nontranslatable exon. Nature (London) **318:**475–477.
32. **Lewis, S., N. Rosenberg, F. Alt, and D. Baltimore.** 1982. Continuing kappa-gene rearrangement in a cell line transformed by Abelson murine leukemia virus. Cell **30:**807–816.
33. **Libermann, T. A., M. Lenardo, and D. Baltimore.** 1990. Involvement of a second lymphoid-specific enhancer element in the regulation of immunoglobulin heavy-chain gene expression. Mol. Cell. Biol. **10:**3155–3162.
34. **Mason, J. O., G. T. Williams, and M. S. Neuberger.** 1985. Transcriptional cell type specificity is conferred by an immunoglobulin V_H gene promoter that includes a functional consensus sequence. Cell **41:**479–487.
35. **Nelsen, B., L. Hellman, and R. Sen.** 1988. The NF-κB-binding site mediates phorbol ester-inducible transcription in nonlymphoid cells. Mol. Cell. Biol. **8:**3526–3531.
36. **O'Neill, E. A., C. Fletcher, C. R. Burrow, N. Heintz, R. G. Roeder, and T. J. Kelly.** 1988. Transcription factor OTF-1 is functionally identical to the DNA replication factor NF-III. Science **241:**1210–1213.
37. **Perez-Mutul, J., M. Macchi, and B. Waslyk.** 1988. Mutational analysis of the contribution of sequence motifs within the IgH enhancer to tissue specific transcriptional activation. Nucleic Acids Res. **16:**6085–6096.
38. **Peterson, C. L., and K. L. Calame.** 1987. Complex protein binding within the mouse immunoglobulin heavy-chain enhancer. Mol. Cell. Biol. **7:**4194–4203.
39. **Peterson, C. L., S. Eaton, and K. Calame.** 1988. Purified μEBP-E binds to immunoglobulin enhancers and promoters. Mol. Cell. Biol. **8:**4972–4980.
40. **Peterson, C. L., K. Orth, and K. L. Calame.** 1986. Binding in vitro of multiple cellular proteins to immunoglobulin heavy-chain enhancer DNA. Mol. Cell. Biol. **6:**4168–4178.
41. **Reik, W., G. Williams, S. Barton, M. Norris, M. Neuberger, and M. A. Surani.** 1987. Provision of the immunoglobulin heavy chain enhancer downstream of a test gene is sufficient to confer lymphoid-specific expression in transgenic mice. Eur. J. Immunol. **17:**465–469.
42. **Scheidereit, C., A. Heguy, and R. Roeder.** 1987. Identification and purification of a human lymphoid-specific octamer-binding protein (OTF-2) that activates transcription of an immunoglobulin promoter *in vitro*. Cell **51:**783–793.
43. **Schlokat, U., D. Bohmann, H. Schöler, and P. Gruss.** 1986. Nuclear factors binding specific sequences within the immunoglobulin enhancer interact differentially with other enhancer

elements. EMBO J. **5**:3251–3258.
44. **Schmidt, E. V., P. K. Pattengale, L. Weir, and P. Leder.** 1988. Transgenic mice bearing the human c-myc gene activated by an immunoglobulin enhancer: a pre-B-cell lymphoma model. Proc. Natl. Acad. Sci. USA **85**:6047–6051.
45. **Scholer, H. R., and P. Gruss.** 1985. Cell type-specific transcriptional enhancement *in vitro* requires the presence of trans-acting factors. EMBO J. **4**:3005–3013.
46. **Sen, R., and D. Baltimore.** 1986. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell **46**:705–716.
47. **Singh, H., R. Sen, D. Baltimore, and P. A. Sharp.** 1986. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. Nature (London) **319**:154–158.
48. **Sive, H. L., and R. G. Roeder.** 1986. Interaction of a common factor with conserved promoter and enhancer sequences in histone H2B, immunoglobulins, and U2 small nuclear (snRNA) genes. Proc. Natl. Acad. Sci. USA **83**:6382–6386.
49. **Staudt, L. M., R. G. Clerc, H. Singh, J. H. LeBowitz, P. A. Sharp, and D. Baltimore.** 1988. Cloning of a lymphoid-specific cDNA encoding a protein binding the regulatory octamer DNA motif. Science **241**:577–580.
50. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. A lymphoid-specific protein binding to the octamer motif in immunoglobulin genes. Nature (London) **323**: 640–643.
51. **Storb, U., C. Pinkert, B. Arp, P. Engler, K. Gollahon, J. Manoz, W. Brady, and R. L. Brinster.** 1986. Transgenic mice with μ and κ genes encoding anti-phosphorylcholine antibodies. J. Exp. Med. **164**:627–641.
52. **Sturm, R., T. Baumruker, B. R. Franza, Jr., and W. Herr.** 1987. A 100-kd HeLa cell octamer binding protein (OBP/100) interacts differently with two separate octamer-related sequences within the SV40 enhancer. Genes Dev. **1**:1147–1160.
53. **Tsao, B. P., X. 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.
54. **Weinberger, J., D. Baltimore, and P. Sharp.** 1986. Distinct factors bind to apparently homologous sequences in the immunoglobulin heavy-chain enhancer. Nature (London) **322**:846–848.
55. **Winoto, A., and D. Baltimore.** 1989. A novel, inducible and T cell-specific enhancer located at the 3′ end of the T cell receptor and locus. EMBO J. **8**:729–733.
56. **Winter, J. N., S. Variakiojis, and A. L. Epstein.** 1984. Phenotypic analysis of established diffuse histocytic lymphoma cell lines utilizing monoclonal-antibodies and cytochemical techniques. Blood **63**:140–146.
57. **Yancopoulos, G. D., and F. W. Alt.** 1985. Developmentally controlled and tissue-specific expression of unrearranged V_H gene segments. Cell **40**:271–281.
58. **Yon, J., and M. Fried.** 1989. Precise gene fusion by PCR. Nucleic Acids Res. **17**:4895.