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The Immunoglobulin Heavy Chain Locus Contains Another B-Cell-Specific 3′ Enhancer Close to the α Constant Region

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The transcription of immunoglobulin genes is controlled by variable region promoters and by enhancers, both of which are lymphoid specific. Because immunoglobulin genes are subject to an extremely complex regulation, we anticipated that there might be additional control elements for these genes. We therefore sought additional enhancers and demonstrate here that there is indeed another weak transcriptional enhancer just 3′ to the mouse α constant region. This novel immunoglobulin enhancer is lymphoid specific and at two positions can bind members of the Oct family of transcription factors.

The immunoglobulin (Ig) genes constitute one of the best-studied systems with respect to cell-type-specific gene expression. Functional Ig genes are created only in B lymphocytes by somatic DNA rearrangements which assemble together, during the early stages of B-cell development, variable (V) and constant (C) region DNA segments (1, 43). Several studies have shown that there is a good correlation between nonproductive (so-called sterile) transcription of the Ig locus (accompanied by hypomethylation) and its first rearrangement (1, 4, 47). In line with this finding, agents which are known to stimulate transcription of the kappa light chain locus also stimulate its DNA rearrangement (35).

Transcription of Ig genes is controlled by V region promoters and by enhancers which are lymphoid specific (3, 11). Originally a strong lymphoid-specific enhancer was identified in the J-C intron of the heavy chain gene (3, 11, 26), and a rather weak enhancer was also found in a similar location in the kappa light chain gene (29, 32). The existence of cell lines which had deleted the heavy chain intron enhancer and yet retained high levels of Ig expression (18, 44) led to the suggestion that the Ig locus could contain other enhancers (14).

Recently new lymphoid-specific enhancers have been discovered 3′ of the kappa gene (22), 3′ of the heavy chain locus (6, 28) and also 3′ of the lambda light chain gene (15). It is generally assumed, but has not been demonstrated, that these various enhancers may each play a role at a different stage of B-cell development.

In spite of this complex picture, it seems likely that the control elements identified thus far do not account for all the regulatory pathways governing Ig gene expression. For example, essentially no information is available about the *cis* elements which control the switch recombination underlying isotype switching (33). However, similar to the situation encountered with the V-J and V-D-J rearrangements, hypomethylation and transcription of the locus seem to accompany or precede the actual recombination (40, 41). Furthermore treatments with certain cytokines can direct switch recombination to particular C regions (21, 46), presumably by activating enhancers which control the sterile transcription of these genes and thus, indirectly, their rearrangement.

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Also, it is possible that the DNA sequences which control the temporally regulated appearance of Igs (heavy chain first and then only light chain [1]) are different from the promoters and enhancers identified thus far. Furthermore, the *cis* elements which control the somatic hypermutation process are not definitively identified.

In an attempt to identify control elements which may be involved in the regulation of switch recombination, we have searched for additional enhancers around the most 3′ C region genes. Here we present evidence that the mouse genome contains another enhancer very close to the Cα gene. This enhancer, which we call the Cα 3′ enhancer, is quite weak and is, like the other Ig enhancers, lymphoid specific. At present, we do not know whether this enhancer is indeed involved in the control of switch recombination to the Cα region. Interestingly, this enhancer has sequences with homology to several sites found in other lymphoid-specific promoters or enhancers, and it binds Oct factors in vitro.

MATERIALS AND METHODS

Plasmids. The J21 reporter plasmid containing the chloramphenicol acetyltransferase (CAT) gene under the control of the c-*fos* promoter has already been described (20, 30, 45). All additional clones were made by inserting DNA fragments (made blunt ended with T4 DNA polymerase) into the *Eco*RV site of J21, 3′ of the CAT gene (ca. 2.5 kb away from the c-*fos* promoter). For some experiments, we also used another CAT reporter plasmid based on the thymidine kinase (TK) promoter (extending up to −105; TK3). In those cases, the cloning site used was a filled-in *Hin*dIII site immediately upstream of the TK promoter. Mouse heavy chain locus DNA fragments were isolated by standard procedures (34) from phage Ch3′Cα12A (16; kindly provided by K. Calame) or from phage Ch.M.Ig ε-12 or Ch.M.Ig γ2b-2 (38; obtained through the Japanese Cancer Research Resources Bank). The control plasmids J21-SV0.17 and TK3-SV0.17 contain a ca. 170-bp simian virus 40 enhancer fragment (recovered from plasmid pBlue-SV by *Eco*RI cleavage) into J21 or TK3. Similarly, the control plasmids J21-Ig0.7 and TK3-Ig0.7 contain the ca. 700-bp *Eco*RI-*Xba*I fragment from the mouse IgH intron enhancer (3, 11).

Cell lines, transfections, and CAT assays. S194 is a mouse B-cell plasmacytoma line, BJA-B is a mature human lymphoblastoid B-cell line, J558 is a mouse myeloma line, HeLa is a human epithelial (cervix carcinoma) cell line, NIH 3T3 is a mouse fibroblast line, BW5147 is a mouse lymphoma line, and U937 is a human macrophage line. Cells (ca. 2×10^7 cells per sample) were transfected with DEAE-dextran followed by a chloroquine boost (2), using either 0.5 or 1.0 μg of DNA per kb of plasmid size (the total amount of DNA was kept constant in each transfection by the addition of sonicated herring sperm DNA).

At 42 to 48 h after DNA addition, protein extracts were prepared by three cycles of freezing-thawing and subsequent incubation at 55°C for 8 min (to inactivate an inhibitory activity present in some lymphoid extracts). CAT assays were set up with equivalent amounts of protein extract (usually 150 to 250 μg; protein amounts were determined with a Bio-Rad protein determination kit and gamma globulin as a standard) and incubated at 37°C for 1 to 2 h, depending on the experiment. The resulting samples were separated on thin-layer chromatography plates, fluorograms of which are presented. For quantitation, the radioactive spots were cut out and counted in a liquid scintillation counter. All transfections experiments were performed a minimum of three times.

DNA sequencing. The originally identified 1-kb *Xba*I fragment was subcloned into the BluescriptKS- vector (Stratagene) to produce pBlue1000+ or pBlue1000−. A series of nested deletions was then generated in these plasmids with enzymes from a commercial kit (Pharmacia), and the resulting plasmids were sequenced on both strands with Sequenase version 2.0 (U.S. Biochemicals).

Nuclear extracts and electrophoretic mobility shifts assays (EMSAs). Nuclear extracts were prepared by a small-scale procedure (37) from various cell lines. Radioactive DNA probes were prepared by digesting plasmid pBlue1000+ (containing the 1-kb *Xba*I 3′ enhancer fragment in BluescriptKS−) with the appropriate restriction enzymes. After dephosphorylation, the fragments were labeled with [γ-^{32}P]ATP and polynucleotide kinase and recovered from polyacrylamide gels. Binding reactions (15 μl) were set up with 4 to 6 μg of protein extract, 5,000 to 10,000 cpm of probe, 2 μg of poly(dI-dC), and 0.3 μg of denatured herring sperm DNA in a buffer containing 4% Ficoll 400, 20 mM *N*-2-hydroxethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.9), 50 mM KCl, 1 mM EDTA, and 0.25 mg of bovine serum albumin per ml. For competition experiments, the conditions were exactly the same as above except that competitor DNA was included in the reaction mixture (in the amounts indicated in the figure legends) before addition of the nuclear extract. The octamer site competitor DNA was a double-stranded oligonucleotide corresponding to nucleotides 533 to 554 of the mouse IgH enhancer (10); the NF-κB competitor DNA was a double-stranded oligonucleotide as described elsewhere (11), and the Cα3′ enhancer competitor DNA was the 1-kb *Xba*I fragment recovered from pBlue1000+. After 10 min at room temperature, the samples were loaded onto prerun 4% polyacrylamide gels containing 22.5 mM Tris-borate and 0.5 mM EDTA (0.25× TBE). Fluorograms of dried gels are presented.

In vitro translation reactions were set up with micrococcal nuclease-treated reticulocytes lysates (Promega) according to the manufacturer's instructions. The RNAs used to program the lysates were obtained by transcribing linearized pBS-Oct-1 (42) or pGem-C+ (23; used to obtain Oct-2 RNA). About 1/20 of each in vitro translation was used for the EMSA.

Nucleotide sequence accession number. The sequence has been deposited in the EMBL data base under accession number Z14251.

RESULTS

Identification of an enhancer immediately 3′ of the Cα region. We have used transient transfection assays into B cells to search for a novel transcription enhancer in the heavy chain locus. Fragments from λ phages containing DNA from the mouse heavy chain locus were inserted into the reporter plasmid J21, in which the CAT gene is driven by the *fos* promoter (sequences from −71 to +109 [20, 30]). This plasmid responds well to stimulation by an enhancer and has been used previously, for example to identify a T-cell receptor α chain enhancer (45). A few initial experiments were made with DNA fragments from around the ε and γ2b constant regions (phages Ch.M.Ig ε-12 and Ch.M.Ig γ2b-2 [38]), but no fragment that could increase CAT expression significantly in transfected B cells was identified. We then concentrated our analysis on a phage containing mouse DNA from around the Cα region and extending for ca. 10 kb further downstream. Three DNA fragments from this phage were able to reproducibly stimulate CAT expression from the reporter plasmid when introduced into S194 mouse plasmacytoma B cells (Fig. 1A): a ca. 7-kb *Eco*RI fragment (J21-7.0 E), a 2.8-kb *Hin*dIII fragment (J21-2.8 H), and a 1-kb *Xba*I fragment (J21-1.0 XXa or J21-1.0 XXb). These three fragments are overlapping, and as shown in Fig. 1A, the 1-kb *Xba*I fragment stimulates CAT expression in either orientation (reproducibly somewhat better in orientation b than in orientation a). Furthermore, these three overlapping fragments also stimulated CAT expression in other B cell lines such as X63Ag8, BJA-B, and J558, while the other DNA fragments from this phage had no stimulatory effect on CAT expression in any of the cell lines tested (Fig. 1 and data not shown). To further substantiate these data, some of these mouse DNA fragments were also tested in a reporter plasmid in which the CAT gene is under the control of the TK promoter. As is shown in Fig. 1B, the 2.8-kb *Hin*dIII fragment and the 1-kb *Xba*I fragment (TK3-2.8 H and TK3-1.0 XX) both stimulated CAT activity, while the previously negative 3.0-kb *Hin*dIII fragment (TK3-3.0 H) was still negative. This result indicates that the effects observed were not promoter specific. In additional experiments using the TK-based reporter plasmid, it was also apparent that the activity of the 7-kb *Eco*RI fragment (used in Fig. 1A) was partially dependent on its orientation (not shown).

To further define the region of the 1-kb *Xba*I fragment important for activity, we tested a number of subfragments for the ability to increase CAT expression from the J21 plasmid. As shown in Fig. 2, a 0.68-kb *Bgl*I-*Xba*I fragment (J21-0.68 BX) as well as a 0.6-kb *Xba*I-*Rsa*I fragment (J21-0.6 XR) both retained most of the enhancer activity in S194 cells. As these two fragments are overlapping, they define the central region of the *Xba*I fragment as the one containing most of the activity. Accordingly, a central *Sty*I-*Xho*II 310-bp fragment (J21-0.31 SXh) still had significant enhancer activity, which increased somewhat when the fragment was duplicated (J21-0.31 SXh_{2x}). However, the slightly higher activity of the complete *Xba*I fragment indicates that the flanking regions are needed for maximal activity.

The 1-kb *Xba*I fragment stimulates CAT activity between 6- and about 20-fold above the background of the J21 or TK3

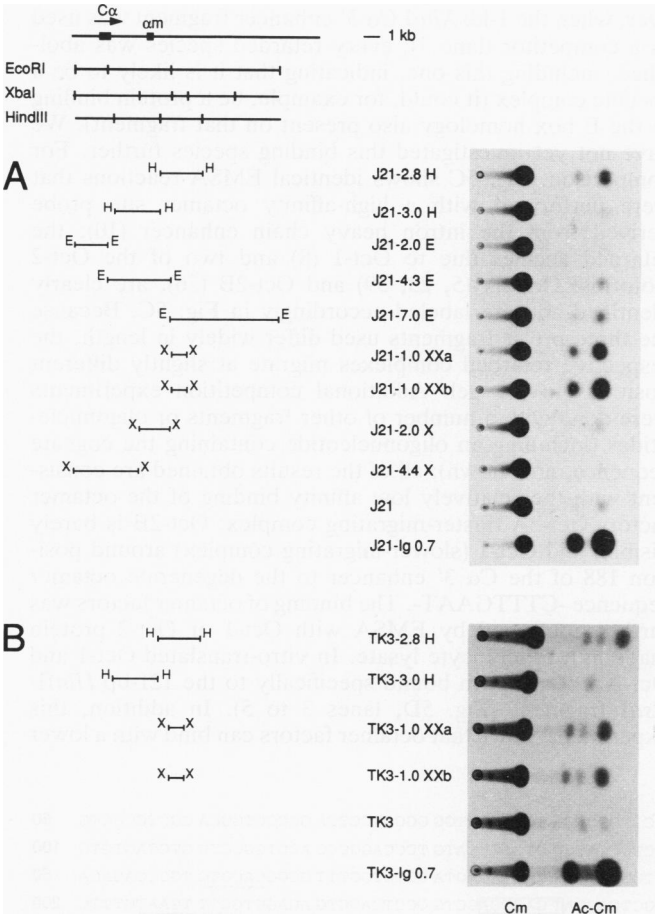

FIG. 1. Identification of an enhancer 3′ of the Cα region. Various restriction fragments from around the Cα region (recovered from phage Ch3′Cα12A [16]) were inserted into the J21 reporter plasmid (A) or into the TK3 reporter plasmid (B). The resulting constructs were introduced into S194 plasmacytoma cells. Subsequently, equivalent amounts of cell extracts were assayed for the ability to convert [^{14}C]chloramphenicol (Cm) to the acetylated form (Ac-Cm). The bar underneath the map of the Cα locus represents the portion of genomic DNA which is present in phage Ch3′Cα12A (16) (the leftmost and rightmost *Eco*RI sites are derived from the cloning procedure). E, *Eco*RI; X, *Xba*I; H, *Hin*dIII. In the plasmid names, the suffixe a or b refers to either orientation of the inserted fragment.

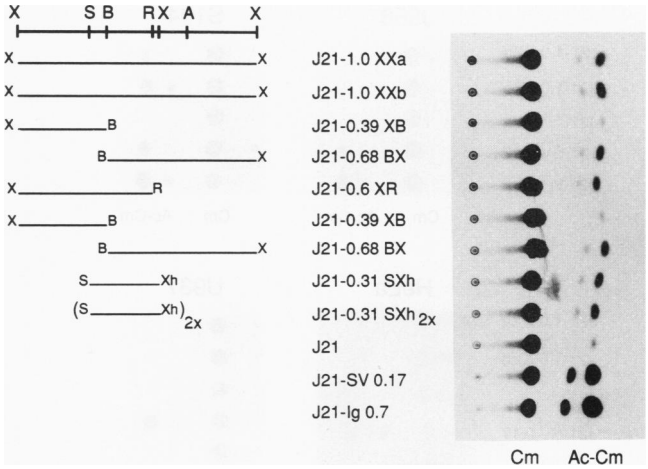

FIG. 2. Fine structure mapping of the Cα 3′ enhancer to delineate the active region of the enhancer. The top bar represents the 1-kb *Xba*I fragment. Subfragments of this *Xba*I fragment were assayed for enhancer activity in S194 cells as described in the legend to Fig. 1. X, *Xba*I; S, *Sty*I; B, *Bgl*I; R, *Rsa*I; Xh, *Xho*II; A, *Acc*I. For J21-0.39 XB and J21-0.68 BX, the results obtained with two independent plasmid isolates are presented. Cm, [^{14}C]chloramphenicol; Ac-Cm, acetylated chloramphenicol.

vector, and this level represents under our experimental conditions ca. 5 to 15% of the stimulation obtained with the IgH intron enhancer. We call this novel Ig 3′ enhancer the Cα 3′ enhancer.

The Cα 3′ enhancer appears to be B cell specific, as it is active (to various degrees) in several B-cell lines (S194, BJA-B, J558, and X63Ag8) but not in HeLa, Cos-7, U937, NIH 3T3, or BW5147 cells (Fig. 3 and data not shown). Note that for HeLa cells, data obtained with both the J21 and TK3 reporter plasmids are presented; in neither case can any activity from the *Xba*I fragment be detected above the basal level of the reporter plasmid (Fig. 3). Because this enhancer is rather weak, we considered the possibility that it might be inducible and would show maximal activity only in the presence of the appropriate inducer(s), in analogy to the κ light chain intron enhancer, which in pre-B cells is inducible by bacterial lipopolysaccharide (LPS) (20). We therefore transfected B-cell lines with the Cα 3′ enhancer constructs J21-1.0 XXa and J21-1.0 XXb as well as with controls and treated the cells with agents such as LPS, phorbol 12-myristate 13-acetate, interleukin-4, or transforming growth factor β. However, none of these agents, either singly or in combination, significantly increased the activity of the Cα 3′ enhancer in any of the cell lines tested (data not shown).

The location of this new enhancer, close to the Cα gene, suggested that it might be involved in controlling switch recombination to Cα, perhaps by activating sterile transcription of the region. In that scenario, the Cα 3′ enhancer could be more active in cells which switch to the Cα region, because they would contain a nuclear factor (or a combination of factors) which activate it. To test that hypothesis, we used two cell clones derived from I29, a cell line which can undergo, upon stimulation with LPS, switch recombination in culture mostly to Cα and to a lesser extent to Cε (40, 41). The first clone, 22D, is a variant which does not switch and makes the μ isotype; the second clone, BFO-3, has switched to Cα and stably makes the α isotype. We transiently introduced the J21-1.0 XXa and J21-1.0 XXb plasmids into these two cell lines and determined the resulting activity. Despite the low transfection efficiency of these cells, it was apparent that the Cα 3′ enhancer was not significantly more active in BFO-3, the IgA-producing line, than in the μ-producing line 22D (not shown).

Sequence of the Cα 3′ enhancer and protein binding analysis. We determined the nucleotide sequence of the central *Sty*I-*Acc*I 447-bp fragment, which has most of the enhancer activity (Fig. 4). Inspection of the sequence revealed several potentially interesting motifs. In particular, three sequences with homology to the octamer site (7, 27) are present. The first one, around position 188 (-CTTTGAAT-), is in the same orientation as the octamer site in the intron heavy chain enhancer (3, 10, 11) and has a 6-of-8-bp match to the consensus -ATTTGCAT- (Fig. 3). The second and the third, at positions 292 and 415, respectively (lower-strand numbering), are both in the opposite orientation and also have a 6-of-8-bp match (-CTGCAAAC-; Fig. 4).

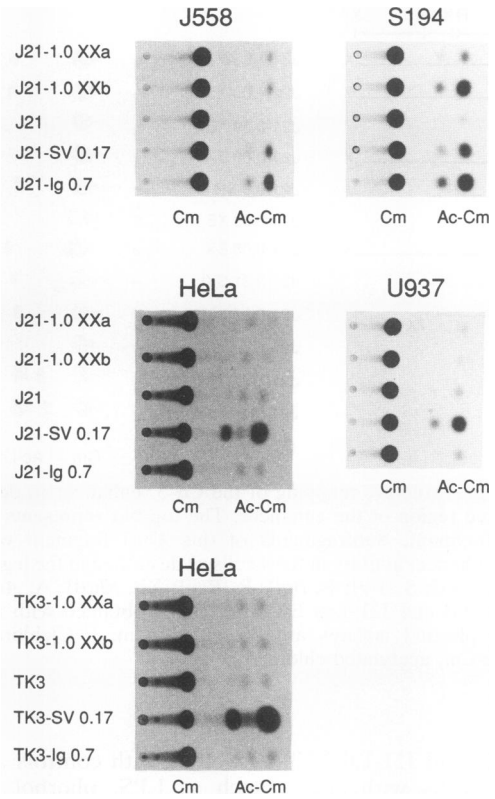

FIG. 3. Cell specificity of the Cα 3′ enhancer. Plasmids were transfected into B-cell (J558 and S194) and non-B-cell (HeLa and U937) lines, and extracts were assayed as described in the legend to Fig. 1. For HeLa cells, results obtained both with the J21 and TK3 reporter plasmids are presented. Cm, [^{14}C]chloramphenicol; Ac-Cm, acetylated chloramphenicol.

Several homologies to the μE5 and κE2 motifs (24), which are binding proteins of the helix-loop-helix family (24, 25), are also found, around positions 142 (lower strand), 201, and 323. Also, a perfect binding site for the AP-1 family of transcription factors (9) is found at position 160 (Fig. 4).

To get a preliminary picture of the nuclear factors that are able to bind on the central portion of the Cα 3′ enhancer, we prepared overlapping fragments from the *Sty*I-*Acc*I fragment and used them in EMSAs. As a source of extract, we used mostly BJA-B cells, because extracts of good quality are easily prepared from them and because the Cα 3′ enhancer is active in these cells. While essentially every fragment tested was to a certain extent shifted in the presence of extract, some of the fragments gave a pattern of retarded bands which was consistent with the binding of octamer factors and were thus analyzed further. Two overlapping fragments, a 199-bp *Hph*I-*Hph*I fragment (Fig. 5A) and a 121-bp *Hin*fI-*Rsa*I fragment (Fig. 5B), both gave rise to two major retarded species in the presence of B-cell extract (lanes 2). Addition of an increasing amount of an oligonucleotide containing an octamer site derived from the IgH intron enhancer efficiently abolished the binding (lanes 3 and 4), while addition of an oligonucleotide containing an NF-κB site (11) did not affect the EMSA pattern (lanes 5 and 6). With the 121-bp *Hin*fI-*Rsa*I fragment (Fig. 5B), an additional retarded species was also clearly observed (running between the two other major species), which was not affected by addition of the competitor DNAs mentioned above. However, when the 1-kb *Xba*I Cα 3′ enhancer fragment was used as a competitor (lane 7), every retarded species was abolished, including this one, indicating that it is likely to be a specific complex (it could, for example, be a protein binding to the E box homology also present on that fragment). We have not yet investigated this binding species further. For comparison, Fig. 5C shows identical EMSA reactions that were performed with a high-affinity octamer site probe derived from the intron heavy chain enhancer (10); the retarded species due to Oct-1 (8) and two of the Oct-2 isoforms, Oct-2A (5, 23, 39) and Oct-2B (36), are clearly identified and are labeled accordingly in Fig. 5C. Because the three probe fragments used differ widely in length, the respective retarded complexes migrate at slightly different positions in the gel. Additional competition experiments were done with a number of other fragments or oligonucleotides (including an oligonucleotide containing the cognate sequence, not shown); all of the results obtained are consistent with the relatively low affinity binding of the octamer factors Oct-2A (faster-migrating complex; Oct-2B is barely visible) and Oct-1 (slower-migrating complex) around position 188 of the Cα 3′ enhancer to the degenerate octamer sequence -CTTTGAAT-. The binding of octamer factors was further confirmed by EMSA with Oct-1 or Oct-2 protein made in a reticulocyte lysate. In vitro-translated Oct-1 and Oct-2 proteins both bound specifically to the 121-bp *Hin*fI-*Rsa*I fragment (Fig. 5D, lanes 3 to 5). In addition, this experiment shows that octamer factors can bind with a lower

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CCTTGGGTGA TGGGGGTTGG GGCCTCCTCA GCCCCTGGCA GGCTCCCCTG    50
GCTCCCACCC CTCATCCATG TCCCAGGCCC ACCTGGCCTG GTCCAGTGTG   100
ATGTGATTCT CAGAACAGTA GCTCTGGTTT GGGGCACCTG TGCTGAGACA   150
GGCTCAGGAT GACTCAGCTG CCCTCAGCTG AGAGCTGCTT TGAATGTTTC   200
AGCAGGTGAT AGACAACAGA GACTTCAGAA GAGAGAAAAA CAAGTTGCTA   250
ATGTGAGCAT CCCTGCCCTA CCCCCACACC TGTACTGCAA ACATTGTTGA   300
CCCCAGATAG AGATCCCAGG ACAGCAGGTG ATAGACAAAG GAGGCTCCAG   350
AGGAGAGAAA AATAGTATCT ATAAGCATGA CTACCTCTGC CCTGCCCCAC   400
ACCTGCCCTG CAAACCTCCC TACGATGCTG ACCCCACATC TGTAGAC      447
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Homologies

Octamer

Cα3'enh	$_{188}$CTTTGAAT
consensus	ATTTGCAT
Cα3'enh inverse	$_{292}$GTTTGCAG
	$_{415}$GTTTGCAG

AP-1

Cα3'enh	$_{160}$TGACTCA
consensus	TGACTCA

μE5

IgH intron	TGCAGGTGT
Cα3'enh inverse	$_{142}$CACAGGTGC

κE2

Cα3'enh	$_{201}$AGCAGGTGA
κ intron	GGCAGGTGG
Cα3'enh	$_{323}$AGCAGGTGA

FIG. 4. Nucleotide sequence of the Cα 3′ enhancer 447-bp central *Sty*I-*Acc*I fragment (the location of this fragment is indicated in the diagram in Fig. 2). Regions with homologies to known motifs are highlighted with dotted lines (κE2 and μE5 sites), solid lines (octamer sites), or a box (AP-1 site). Under the sequence, the homologies are compared with canonical sites, with identities indicated by dots. Note that in some cases (labeled inverse), the sequence of the lower strand is indicated with the numbering adjusted accordingly.

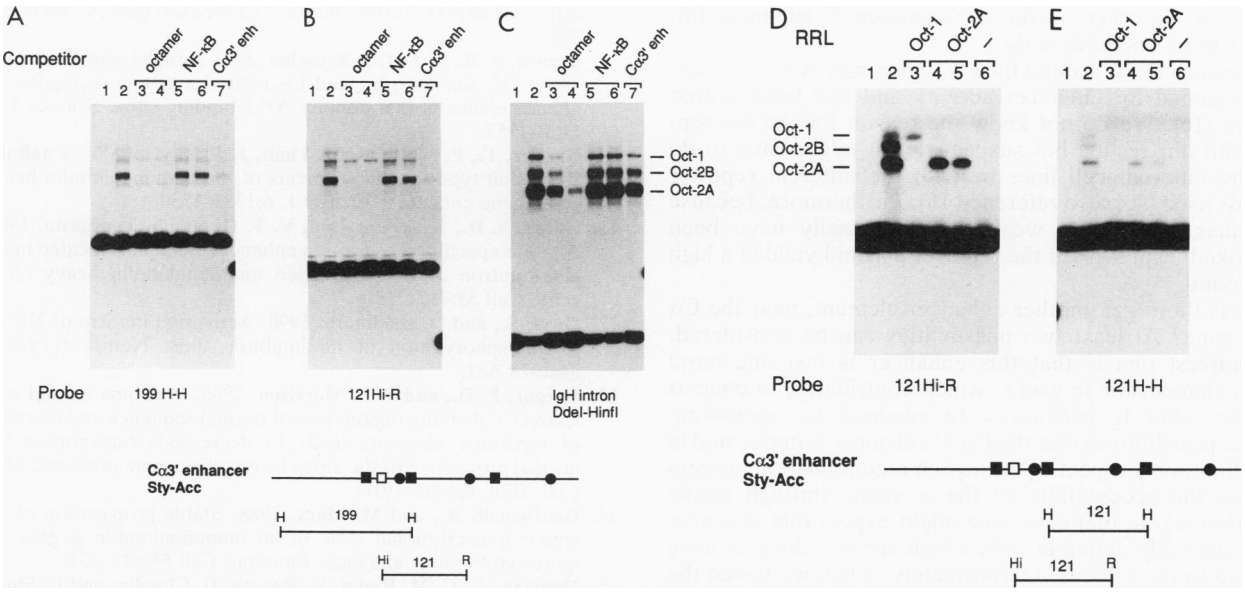

FIG. 5. In vitro binding of octamer factors to the Cα 3′ enhancer *Sty*I-*Acc*I fragment. (A to C) EMSAs with BJA-B nuclear extract and the following labeled DNA fragments as probes: a 199-bp *Hph*I-*Hph*I fragment (199 H-H) (A), a 121-bp *Hin*fI-*Rsa*I fragment (121 Hi-R) (B), and a 47-bp *Dde*I-*Hin*fI fragment (containing a perfect octamer site) (C) from the mouse intron heavy chain enhancer (10). Lanes: 1, no nuclear extract; 2 to 7, BJA-B (B-cell) nuclear extract. In lanes 3 to 7, various competitor DNAs were added, the nature of which is indicated above the lanes. The amounts of specific competitor DNA added were as follows: lanes 3 and 5, 100 fmol; lanes 4 and 6, 500 fmol (about a 50-fold excess); and lanes 7, 450 fmol. The diagram depicts the *Sty*I-*Acc*I fragment. The positions of homologies to known motifs are indicated as follows: solid circles, octamer sites; solid squares, κE2 and μE5 sites; and open square, AP-1 site. Underneath are indicated the positions of the restriction sites used as well as the lengths (in base pairs) of the resulting fragments. H, *Hph*I; Hi, *Hin*fI; R, *Rsa*I. (D and E) EMSAs with reticulocytes lysates or B-cell extracts, using a 121-bp *Hin*fI-*Rsa*I probe (121 Hi-R) (D) and a 121-bp *Hpa*I-*Hpa*I probe (121 H-H) (E). Lanes: 1, no extract or reticulocyte lysate added; 2, BJA-B nuclear extract; 3 to 6, rabbit reticulocyte lysate (RRL). The origin of the RNA used to program the lysate is indicated above each lane.

affinity at a second position on the Cα 3′ enhancer. Figure 5E shows that the 121-bp *Hpa*I-*Hpa*I fragment binds octamer factors (from BJA-B extract [lane 2] or in vitro translations [lanes 3 to 5]) with very low affinity. Here too, extensive competition experiments verified the identity of the complexes observed (not shown).

The fact that the two octamer sites identified are in relative proximity (slightly less than 100 bp apart [Fig. 4]) raised the possibility of cooperative binding on the natural fragment, in particular because it had previously been shown that octamer factors can bind cooperatively to their cognate sites in certain cases (17, 19, 31). We therefore prepared as a probe a restriction fragment which contained both sites; however, EMSA analysis failed to reveal cooperative binding. The third octamer site homology at position 408 does not appreciably bind octamer factors under our conditions (data not shown), even though it has a sequence identical to the sequence of the site at position 285. Therefore, the context of these two sites (flanking sequences) must account for their difference in binding capacity.

We have not yet further analyzed what other factors specifically bind to the Cα 3′ enhancer; this analysis requires additional work.

DISCUSSION

In this report, we have presented evidence that the mouse genome contains another 3′ enhancer very close to the Cα region. This enhancer is different from the recently identified 3′ enhancer which is ca. 25 kb downstream of the Cα gene in the rat genome (28) and ca. 16 kb downstream of it in the mouse genome (6).

Analysis of this Cα 3′ enhancer in several cell lines demonstrated that it was lymphoid specific, like the other Ig enhancers, but quite weak. Under the conditions that we used, it did not appear to be inducible. DNA sequence analysis of the central region revealed sequences with homology to several known motifs which have also been found in other Ig enhancers: octamer-like sites, κE2/μE5-like sites, and also one perfect AP-1 site. In vitro binding analysis with nuclear extracts showed that two of the three octamer site homologies can bind their cognate factors with relatively low affinity. Moreover, preliminary results suggest a functional importance for these sites, because their mutation somewhat reduces the activity of the Cα 3′ enhancer in transfected S194 cells (data not shown). Clearly other factors also bind on the Cα 3′ enhancer, but they have not yet been analyzed further.

Previous studies had shown by run-on analysis that myeloma cells which had spontaneously deleted the region immediately 3′ of the Cα gene (including the membrane exon and encompassing the region where the Cα 3′ enhancer is located) had a severalfold-decreased rate of alpha gene transcription (13). It had then been suggested that these deleted sequences could have contained transcription-enhancing sequences, or else that the newly juxtaposed sequences could have contained transcription-inhibiting sequences (13). In light of our results, it now appears likely that the former hypothesis was correct, and this observation suggests that the Cα 3′ enhancer probably has a biological role. However, the presence or absence in these myeloma cells of the Cα 3′ enhancer (as well as the presence or

absence of the other, further-downstream 3′ enhancer [6]) remains to be tested directly.

The mouse DNA around the Cα region had in fact already been screened for enhancer activity and had been scored negative (16). We do not know the reason for the discrepancy with our results but suspect that it might have to do with the different cell lines or with the different reporter plasmids used (see also reference 16). Furthermore, because this enhancer is quite weak, it could easily have been overlooked, especially if the reporter plasmid yielded a high background.

Why is there yet another enhancer element, near the Cα region gene? At least two possibilities can be considered. The simplest one is that this enhancer is just one more control element for Ig genes, which contributes, in concert with the other Ig enhancers, to maximal Ig expression. Another possibility is that the Cα 3′ enhancer is implicated in controlling some aspect of the switch recombination process (perhaps the accessibility of the α locus, through sterile transcription). In that case, one might expect this enhancer to be maximally active in cells which are switching or have switched to the α locus. Unfortunately, when we tested the Cα 3′ enhancer in two cell lines (one of the α isotype and the other of the μ isotype) derived from the same parental line, we did not observe a significant difference in activity. However, one should bear in mind that the activation of such an enhancer could be a transient phenomenon that might escape detection by the use of cell lines. Definitive evaluation of the role of this enhancer for Ig expression awaits experiments with transgenic mice or its targeted disruption in the mouse germ line.

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