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Immunology

B-cell-specific enhancer activity of conserved upstream elements of the class II major histocompatibility complex *DQB* gene

(X and Y box sequences/transient expression assay)

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ABSTRACT **A 95-base-pair immediate upstream sequence of the human class II major histocompatibility complex *DQB* gene containing the conserved X and Y elements showed enhancer activity in a transient expression assay. An "enhancer test plasmid" harboring the bacterial chloramphenicol acetyltransferase gene under the control of a truncated simian virus 40 enhancerless early promoter was employed. The *DQB* sequence inserted into this plasmid was active as an enhancer in Raji cells (human Burkitt lymphoma cells) but not active in Jurkat cells (human T-cell leukemia cells) or in HeLa cells (human cervical carcinoma cells). This cell-type specificity suggests that this enhancer activity may be involved in the tissue specificity of the *DQB* gene that is normally expressed only in mature B cells, macrophages, and thymic epithelial cells. Deletion analysis showed that both X and Y box sequences are essential for the full activity of the enhancer sequence and that these two sequences may function in a cooperative manner as cis-acting elements. Further deletions were used to define the 5′ border of the X element. These results suggest that previously characterized protein factors that bind to X and Y include transcription factors involved in the cell-type specificity of this enhancer activity.**

The class II antigens of the major histocompatibility complex are glycoproteins consisting of α and β chains. They play crucial roles in the immune response. The expression of these proteins on the surface of cells is essential for presentation of foreign antigens to T helper cells (1, 2). In humans, at least three species of functional class II antigens, namely DR, DQ, and DP, are expressed. Genes encoding the α and the β chains of those antigens are scattered over 1500 kilobases within the *HLA-D* region on chromosome 6 (3, 4). Expression of these genes is coordinately regulated except in a few cell types where selective expression has been reported (e.g., ref. 5). Constitutive expression of the genes is restricted to mature B cells, macrophages, and thymic epithelial cells. Moreover, in some types of cells such as fibroblasts and endothelial cells, γ-interferon induces expression of the class II genes (6, 7). In addition interleukin 4 (8) and prostaglandin (9) can also affect the expression levels of these genes.

Coordinate expression of the class II genes suggests a common regulatory mechanism(s). Mutant B-cell lines that lack the expression of all class II genes have been isolated by somatic cell mutagenesis (10–12). Cell fusion experiments using these cells have shown that the class II gene expression of these cells can be rescued by trans-acting factor(s) provided by class II-positive mouse B cells (13) and T cells (14). A complementation study using several such mutant cell lines, including one from a patient with class II-negative bare-lymphocyte syndrome, has suggested that there are at least two distinct trans-acting factors specifically required for the expression of the class II genes (15).

Two highly conserved sequences, termed X and Y boxes, occur in the immediate upstream regions of all of class II genes of humans and mice, and their role in the regulation of class II gene expression has been suggested (16, 17). In the *DQB* gene used in this study, the conserved box X is located from −113 to −100 base pairs (bp) relative to the cap site and the conserved box Y is located from −81 to −67 bp (18) (see Fig. 1*A*). A study by gel retardation and DNase I "footprinting" assays has identified several protein factors, some of which are B-cell-specific, in nuclear extracts from various types of cells that bind to the X or Y box sequence of the *DQB* gene (19). Several other studies using the *DRA* gene (20) and the mouse class II E_α gene (21, 22) also detected protein factors binding to the X and Y box sequences of these genes. It is likely that some protein factors detected in these studies are transcriptional factors involved in the regulation of class II gene expression. In fact a short promoter region including X and Y boxes of the *DQB* gene has been shown to respond to γ-interferon induction in human fibroblasts in a DNA transfection experiment (23). However, the roles of X and Y box sequences and the protein factors that bind to these sequences in the tissue specificity of class II gene expression are still unclear, although several relevant studies on class II genes, such as the *DRA* gene (20), *DQB* gene (24), and mouse class II E_β gene (25) and E_α gene (21), have been reported.

To gain some insight on this issue, transient expression assays have been carried out using an "enhancer test plasmid" containing the bacterial chloramphenicol acetyltransferase (CAT) gene fused to a truncated simian virus 40 (SV40) early promoter. We report here that the immediate upstream region of the *DQB* gene including the X and Y box sequences has a B-cell-specific enhancer activity in this assay system. This finding and results of further deletion analysis are discussed in conjunction with data (19) on the nuclear protein factors that bind to the *DQB* sequence.

MATERIALS AND METHODS

DNA and Plasmids. The *DQB* gene and its derivatives were subcloned from λ42, which contained the *DQB2* gene (18). BUG-Ig-cat was kindly provided by Julian Banerji (Harvard University). BUG3cat was derived from BUG-Ig-cat and harbored the *Hin*dIII–*Bam*HI fragment of pSV2cat (26) containing the CAT gene, which was connected to the SV40 early promoter sequence between nucleotides 99 and 5171 of the SV40 genome (27) (see also *Results*). A plasmid harboring human growth hormone (hGH) gene, pϕGH (28), was kindly provided by David D. Moore (Massachusetts General Hospital). The *Mlu* I–*Hin*dIII fragment of pRSVcat (29) containing the Rous sarcoma virus long terminal repeat sequence was inserted with *Hin*dIII linker DNA into the *Hin*dIII site of

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Abbreviations: CAT, chloramphenicol acetyltransferase; SV40, simian virus 40; hGH, human growth hormone.

pφGH to make pRSVGH, and it was used as an internal control for transfection efficiency in DNA transfection experiments.

Cells and Transfections. Raji is a human Burkitt lymphoma cell line; Jurkat is a human T-cell leukemia cell line; HeLa is a human cervical carcinoma cell line. Raji cells are class II-positive, whereas Jurkat and HeLa cells are class II-negative (refs. 30 and 31 and Z. Yang and J.L.S., unpublished data). Raji cells and Jurkat cells were maintained in RPMI 1640 medium with 10% (vol/vol) fetal calf serum. HeLa cells were maintained in Dulbecco's modified Eagle's minimum essential medium (DMEM) with 10% (vol/vol) fetal calf serum. Raji cell and Jurkat cell transfection was carried out by the DEAE-dextran method (32). In brief, 5×10^6 Raji cells were incubated for 1 hr in 4 ml of a DNA/DEAE-dextran mixture containing DMEM, 50 mM Tris·HCl (pH 7.3), DEAE-dextran (50 μg/ml; Pharmacia), 10 μg of test construct DNA, and 2 μg of pRSVGH DNA. Then cells were washed and maintained in RPMI 1640 medium with 10% (vol/vol) fetal calf serum and harvested 48 hr after transfection. Jurkat cell transfection was done in the same manner as above except that the DEAE-dextran concentration was 1.5 mg/ml and cells were harvested 72 hr after transfection. HeLa cells (10^6 cells) were transfected with 20 μg of the test construct DNA and 2 μg of pRSVGH DNA by the calcium phosphate coprecipitation method described by Chen and Okayama (33). Cells were harvested 48 hr after removal of DNA–$CaPO_4$ precipitates.

CAT Assay and hGH Assay. After harvest, cell extracts were prepared from transfected cells and assayed for the CAT activity (26). Incubation time for the CAT assay was 2 hr for Raji cells, 4 hr for Jurkat cells, and 1 hr for HeLa cells. At the time of harvest, medium of the transfected Raji cells and Jurkat cells were assayed for hGH concentration by using a hGH assay kit (Hybritech, San Diego, CA). With HeLa cells, hGH concentration in the cell extracts was measured. All CAT assays were normalized to the hGH concentration, and CAT activity values shown in this paper are averages of at least three independent experiments.

RESULTS

Preliminary DNA transfection experiments using a series of test constructs of various lengths of the *DQB* upstream sequence connected to the CAT gene revealed that a 192-bp DNA sequence from −160 to +32 bp relative to the cap site of the *DQB* gene (Fig. 1*A*) was sufficient to confer a tissue specificity on CAT gene expression (data not shown). This observation prompted a detailed examination of the cis-acting element(s) within this region that had tissue-specific enhancer activity.

The region including both the X and Y box sequences was tested for enhancer activity. A sequence from −160 to −66 bp relative to the cap site of the *DQB* gene (−160/−66 sequence) was excised with the restriction enzymes *Aat* II and *Fok* I. After filling in and adding *Xba* I linkers, it was ligated into the *Xba* I site of a plasmid, BUG3cat (Fig. 1*B*), in each orientation to make two test constructs. The −160/−66 sequence contained both X and Y box sequences and the sequence upstream to them but did not contain the putative CCAAT sequence, "TATA box" sequence, or cap site of the *DQB* gene (18). BUG3cat contained the CAT gene under the control of a truncated SV40 early promoter in which 72-bp repeat sequences (but not the 21-bp repeats) were removed and an *Xba* I site was located ≈100 bp upstream from the cap site of this promoter between the promoter sequence and the polylinker sequence of the pUC13 vector.

The two test constructs and BUG3cat itself were transfected into Raji cells, Jurkat cells, and HeLa cells. CAT assays were then carried out to see if the presence of the −160/−66

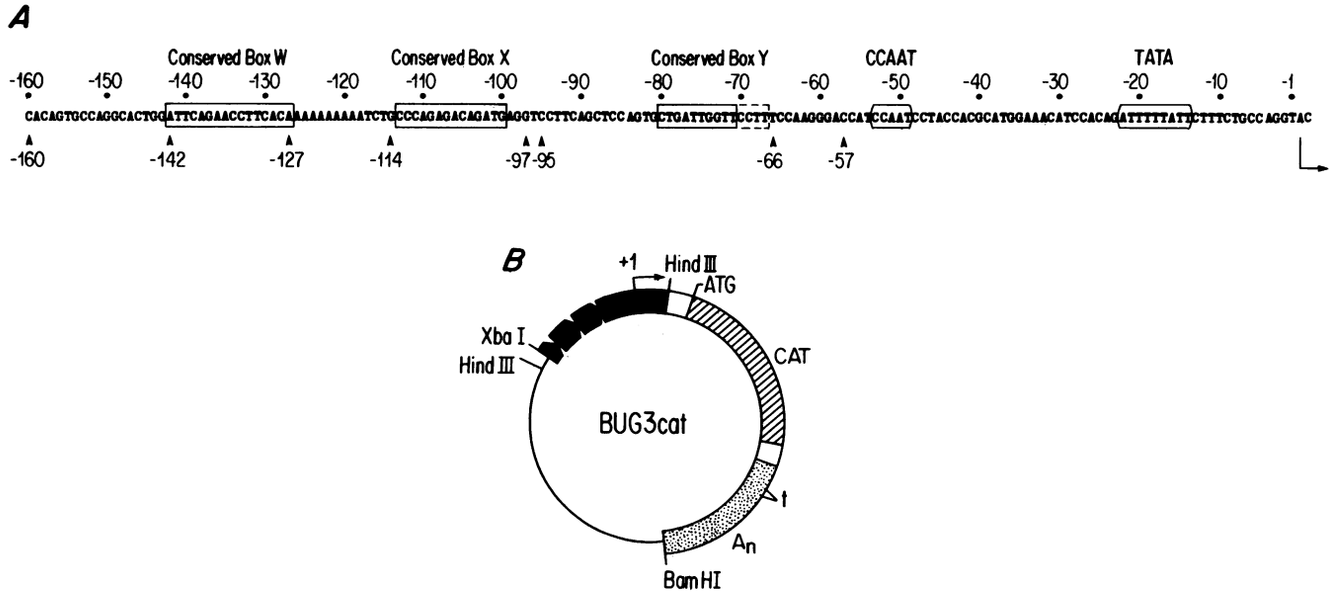

FIG. 1. (*A*) Schematic representation of the *HLA-DQB* gene immediate upstream region (20, 24). TATA box (−22 to −146 bp), "CCAAT box" (−53 to −49 bp), conserved sequence Y (−80 to −67 bp), conserved sequence X (−113 to −100 bp), conserved sequence W (−142 to −127 bp) are indicated. End points of inserts in the test constructs used in this study are indicated by arrowheads. Position of the cytidine residue at the 5′ end of the sequence shown in this figure was −158 bp relative to the cap site as reported (19, 20, 24). However, in this study, an adenosine residue at −159 bp and a cytidine residue at −152 bp were found to have been missed previously. (*B*) Schematic representation of BUG3cat. In BUG3cat used as an enhancer test plasmid in this study, SV40 early promoter sequence (represented by closed box) is truncated within one of the 21-bp repeat sequences (represented by ■) furthest to the cap site (indicated by +1) of this promoter, and connected with an *Xba* I linker to the *Xba* I site of the pUC13 sequence (represented by thin line). The *Hin*dIII–*Bam*HI fragment that contains the CAT coding sequence (hatched box) and SV40 sequence harboring splicing (indicated by t) and polyadenylylation (indicated by An) signals was derived from pSV2cat (27). Position of the initiation codon of the CAT gene is also shown. *Xba* I site and *Bam*HI site were used for inserting the *DQB* sequences into this plasmid.

sequence resulted in enhancement of CAT gene expression level. The three recipient cells had the following properties. Raji cells derived from Burkitt lymphoma express endogenous class II genes. Jurkat cells derived from human T-cell leukemia or HeLa cells from cervical carcinomas do not express the endogenous genes. As shown in Table 1 and Fig. 2, the −160/−66 sequence enhanced CAT expression 21.0-fold in the natural orientation and 3.3-fold in the reverse orientation in Raji cells. By contrast, in Jurkat cells or in HeLa cells, this sequence in either orientation showed no effect on CAT gene expression level (1.0- to 1.6-fold).

To determine whether the −160/−66 sequence can stimulate CAT gene expression from a downstream position, the −160/−66 insert was excised from one of the two test plasmids above with *Xba* I, ligated to *Bam*HI linker DNA, and inserted into the downstream *Bam*HI site of BUG3cat (Fig. 1*B*) in each orientation. In BUG3cat, the *Bam*HI site was located ≈2 kilobases downstream of the transcription start site of the SV40 promoter after the SV40 splice and termination sequences. The activity of these two plasmids was assayed and compared with that of BUG3cat in the three kinds of cells described above. Even when inserted downstream of the CAT gene, the −160/−66 sequence enhanced CAT gene expression 9.5-fold in its natural orientation and 5.2-fold in its reverse orientation in Raji cells (Table 1 and Fig. 2). By contrast, this sequence in either orientation had little effect on CAT gene expression level in Jurkat cells or in HeLa cells (0.9- to 1.6-fold). From these observations, we concluded (*i*) that the −160/−66 sequence had the orientation- and position-independent properties of an enhancer element and (*ii*) that this activity was cell-type specific, in that this sequence was active in a B-cell line but not active in a T-cell or in a cervical carcinoma cell line.

To define the minimum sequence requirement for the enhancer activity, DNA transfection experiments with CAT constructs described below were carried out. First, to see whether an X or Y box sequence alone was sufficient for the enhancer activity or whether both were required, the sequence from −160 to −95 bp (*Aat* II–*Sau*96I) and that from −97 to −57 (*Sau*96I–*Sau*96I) were inserted in each orientation into the *Xba* I site of BUG3cat with *Xba* I linker DNA.

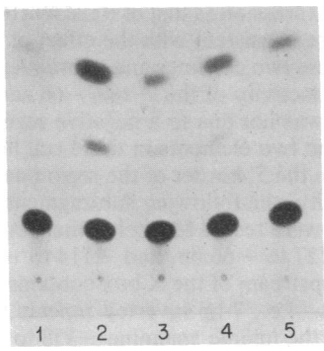

FIG. 2. Enhancement of CAT gene expression by the −160/−66 sequence from the immediate upstream region of the *DQB* gene in Raji cells. Raji cells were transfected with the test plasmids containing the −160/−66 sequence as well as BUG3cat. Extracts from the transfected cells were assayed for CAT activity. The autoradiogram shows relative amounts of acetylated chloramphenicol reaction products separated from unacetylated chloramphenicol on a thin layer chromatogram. Lanes: 1, BUG3cat (no insert); 2, BUG3cat with the −160/−66 sequence at the *Xba* I site (on the 5′ side of the CAT gene) in natural orientation; 3, BUG3cat with the −160/−66 sequence at the *Xba* I site in reversed orientation; 4, BUG3cat with the −160/−66 sequence at the *Bam*HI site (on the 3′ side of the CAT gene) in natural orientation; 5, BUG3cat with the −160/−66 sequence at the *Bam*HI site in reversed orientation.

Then CAT expression was tested from these four plasmids in all three cell types. The −160/−95 sequence contained the X box and proximal upstream sequence, and the −97/−57 sequence consisted of Y box and sequences flanking it (see Fig. 1*A*). In Raji cells, the −160/−95 sequence showed much lower activity than the −160/−66 sequence that contains both X and Y boxes—i.e., it enhanced CAT expression only 3.5-fold even in its natural orientation. Furthermore, the −97/−57 sequence showed only marginal effect on CAT expression level (Table 1). Thus both elements appear to be crucial for the full activity of the enhancer element. In Jurkat cells or in HeLa cells, each of the CAT constructs harboring the −160/−95 or the −97/−57 sequence gave the same

Table 1. Relative CAT activity values of test constructs containing DNA sequences from the *DQB* immediate upstream region

BUG3cat insert	Position	Orientation	Relative CAT activity		
			Raji cells	Jurkat cells	HeLa cells
None	—	—	1.0	1.0	1.0
−160 to −66 bp	*Xba* I site	Natural	21.0 ± 5.0	1.6 ± 0.4	1.6 ± 0.8
		Reverse	3.4 ± 0.5	1.0 ± 0.1	1.3 ± 0.1
	*Bam*HI site	Natural	9.5 ± 1.5	0.9 ± 0.2	1.6 ± 1.0
		Reverse	5.2 ± 0.3	0.9 ± 0.1	0.9 ± 0.5
−160 to −95 bp	*Xba* I site	Natural	3.5 ± 1.0	0.8 ± 0.2	1.4 ± 0.1
		Reverse	1.3 ± 0.2	0.8 ± 0.1	1.2 ± 0.3
−97 to −57 bp	*Xba* I site	Natural	1.8 ± 1.0	2.0 ± 0.7	1.1 ± 0.4
		Reverse	1.1 ± 0.2	ND	1.1 ± 0.4

A series of test constructs was made by inserting the *DQB* sequences into BUG3cat at indicated positions (the *Xba* I site on the 5′ side of the CAT gene or the *Bam*HI site on the 3′ side of the CAT gene) and in indicated orientations. Boundaries of the inserts are expressed in base pairs relative to the cap site of the *DQB* gene. The test constructs were transfected into Raji cells, Jurkat cells, and HeLa cells. Cells are harvested after transfection and the cell extracts were assayed for CAT activity. CAT activity of each construct is expressed relative to the BUG3cat result—that is, the CAT activity of BUG3cat is set equal to 1.0 (relative CAT activity). The standard deviation of each relative CAT activity value was calculated by using an electronic calculator according to the formula, $SD = [\Sigma_i^n (X_i - X_{av})^2/(n - 1)]^{1/2}$, where X_i is the observed value, X_{av} is the average, and n is the number of data points. The number of data points for each of the test constructs was 3–11. Radioactivities of the acetylated chloramphenicol reaction products obtained after CAT assays of extracts from cells transfected with BUG3cat were 1000–2000 cpm in Raji cell transfections, 400–900 cpm in Jurkat cell transfections, and 1000–6000 cpm in HeLa cell transfections. In separate experiments, pRSVcat gave CAT activities ≈300-fold higher than that with BUG3cat in Raji cells and in Jurkat cells and ≈1000-fold higher than that with BUG3cat in HeLa cells.

levels of CAT expression as that of BUG3cat (0.8- to 2.0-fold). These data were consistent with the effect of the −160/−66 sequence in these two cell lines and, moreover, indicated that that apparent inactivity of the −160/−66 sequence in these two cell types was not due to a negative regulatory effect of either one of the two elements in these cell lines.

To determine the 5′ border of the region necessary for the enhancer activity, the following subfragments of the −160/−66 sequence were tested for their enhancer activity: −142 to −66 bp, −127 to −66 bp, and −114 to −66 bp. In fact, the sequence upstream of the X box contains two characteristic sequences—i.e., 7-bp inverted repeats with an adenosine residue in the middle spanning −158 to −144 bp and a sequence designated as W box between −140 and −127 bp, the latter previously noted to be conserved among class II β-chain gene upstream sequences (19). These three fragments were inserted with *Xba* I linkers into the *Xba* I site of BUG3cat. The resulting plasmids were assayed for activity in Raji cells. Although −142/−66 and −127/−66 fragments showed levels of enhancement of CAT gene expression (23.4-fold and 14.8-fold, respectively) similar to the −160/−66 sequence in the natural orientation, the −114/−66 sequence showed much lower activity (3.5-fold) than the others (Fig. 3). This result indicated that the region between −127 and −115 bp might play a crucial role in the enhancer activity, at least with respect to the activity of the element in its natural orientation. On the other hand, the enhancer activity varied in a complicated manner in the reverse orientation (Fig. 3). First, the −160/−66 fragment showed a 3.3-fold enhancement of CAT expression in the reverse orientation (see Table 1). Interestingly, in the reverse orientation, enhancement by −142/−66 fragment (15.9-fold) was much higher than that by the −160/−66 fragment. The

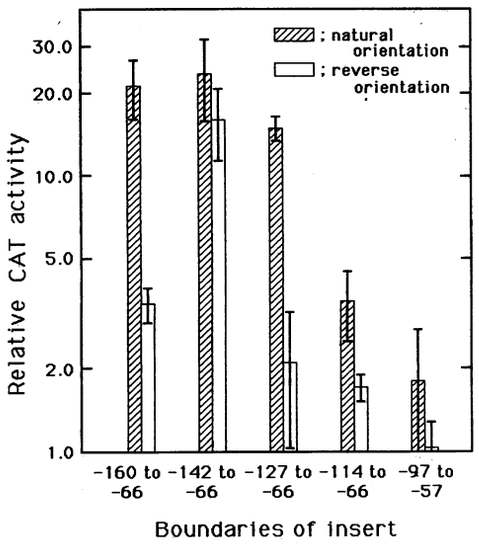

FIG. 3. A 5′ deletion analysis of the −160/−66 sequence. The test constructs with the subfragments derived from −160/−66 sequence, the ranges of which are indicated in this figure, were transfected into Raji cells and CAT activity in the cell extracts was assayed. Boundaries of each subfragment are represented in base pairs relative to the cap site of the *DQB* gene. CAT activity of each construct is expressed relative to the BUG3cat, such that the CAT activity of BUGcat is set equal to 1.0 (relative CAT activity). Hatched bars show relative CAT activity values of the test constructs with inserts at the *Xba* I site (on the 5′ side of the CAT gene) in natural orientation. Open bars show relative CAT activity values of the test constructs with inserts at the *Xba* I site in reverse orientation. Relative CAT activity values are shown on a logarithmic scale. Standard deviation of these values is indicated by error bars. Data on the test constructs with −97/−57 sequence are also included in this figure for comparison.

−127/−66 fragment showed little activity (2.1-fold) and the −114/−66 bp fragment gave no enhancement (1.0-fold). The inverted repeats between −158 and −144 bp and W box sequence between −140 and −127 bp may have some effect on the enhancer activity in the reverse orientation.

DISCUSSION

In this study, a 95-bp sequence (−160 to −66 bp relative to the cap site) within the upstream region of the *DQB* gene has been shown to act as an enhancer in Raji cells when tested in an "enhancer test plasmid" containing the CAT gene under the control of a truncated SV40 early promoter. This sequence was active in Raji cells but inactive in Jurkat cells or in HeLa cells, suggesting that this enhancer activity is involved in the tissue-specific expression of the *DQB* gene. This does not exclude the possibility of additional tissue-specific cis-acting element(s) outside this region (20, 24, 25).

Several enhancer elements within or around various viral and cellular genes have been characterized (34, 35) and some of them, such as immunoglobulin gene enhancers, have been demonstrated to be responsible for the tissue specificity of gene expression (32, 36, 37). Furthermore, studies on genes, such as the metallothionein genes (38), *Xenopus* heat shock protein (hsp70) gene (39), and major histocompatibility complex class I genes (40, 41), have revealed that immediate upstream regions (≈300 bp to ≈60 bp upstream to cap site) of these genes can act as enhancers or enhancer-like elements when introduced into test plasmids containing heterologous promoters. In such regions short conserved sequences, such as metal-responsive elements (ref. 38 and references therein) and heat shock elements (ref. 39 and references therein) have been identified as immediate upstream cis-acting elements and then as binding sites for specific transcriptional factors involved in tissue specificity and/or inducibility of the gene expression.

The −160/−66 sequence contains two short conserved sequences designated as X and Y boxes from −113 to −100 bp and from −80 to −67 bp, respectively. An extensive study by gel retardation and DNase I footprinting assays (19) has revealed that both X and Y box sequences of the *DQB* gene are binding sites for several protein factors in nuclear extracts from various types of cells. The enhancer activity of the −160/−66 sequence and the observations that sequences upstream of −128 bp could be deleted without significant loss of activity [at least when sequences were tested upstream of the promoter (Fig. 3)] suggest that some of the nuclear protein factors that bind to X and/or Y box sequences are in fact transcriptional factors responsible for the tissue specificity of this enhancer element. That X and Y box sequences are quite well conserved among class II genes and that binding of nuclear factors to these sequences in class II genes other than the *DQB* gene has also been detected (21, 22) further indicate that the immediate upstream regions of other class II genes that include X and Y boxes are similar tissue-specific enhancers.

Separation of the X and Y boxes in the −160/−66 sequence greatly reduced the activities of the resulting two fragments in Raji cells as compared with that of the intact −160/−66 sequence. The −160/−95 fragment was less active by about a factor of 6 than the −160/−66 sequence, and the −97/−57 sequence had little activity when tested upstream of the CAT gene in the natural orientation. This result suggests the following two possibilities: (*i*) both X and Y box sequences play significant roles in a cooperative manner in the enhancer activity as binding sites for transcriptional factors, or (*ii*) dissection at the *Sau*96I site of the −160/−66 sequence may simply disrupt a cis-acting element that some transcriptional factor(s) recognizes and/or binds to. The first possibility seems more likely than the second because DNase I footprinting experiments (ref. 19 and C. F.

Doyle and J.L.S., unpublished data) could detect only a modest level of protection within the sequence between X and Y boxes only when high concentration of Raji cell nuclear extract was used. Moreover, because of *Xba* I linker sequences (CTCTAGAG) at both ends of the inserted sequences, the insert in −160/−95 construct is in fact identical to that of the *DQB* upstream between −160 and −93 bp. For the same reason, the insert in −97/−57 construct can be regarded as containing the *DQB* sequence from −100 to −56 bp, which means that a guanosine residue at the 3′ end of the X box in addition to all the sequence between the X and Y boxes is included in this insert (Fig. 1*A*). Therefore, if any transcriptional factor could bind to a region just downstream of the X box and if it played a significant role in the enhancer activity, the −97/−57 sequence should have shown enhancer activity. These arguments lead us to a tentative conclusion that cooperative interaction between transcriptional factors that bind to X and Y boxes, respectively, may be crucial for the activity of this enhancer element. The fact that the distance between X and Y boxes is also well conserved among upstream sequences of class II genes (16, 17) supports this notion.

The Y box contains a CCAAT sequence in the reverse orientation. CCAAT sequences have been found, often in reverse orientation, and identified as cis-acting elements within the immediate upstream regions of a number of viral and cellular genes. Specific transcriptional factors have been demonstrated to bind to this sequence, some of which have been purified and characterized (refs. 22, 42, and 43 and references therein). Thus, results in this study imply that transcriptional factor(s) that binds to the CCAAT sequence in the Y box can play a significant role in the enhancer activity, presumably in association with other factor(s) that recognize the X box. In this regard, it is of interest to note here that the upstream sequence of *Xenopus* heat shock protein (hsp70) gene that acts as an inducible enhancer element contains a CCAAT sequence in reverse orientation between two heat shock elements, although its function has not yet been tested (39).

The 5′ deletion analysis of the −160/−66 sequence revealed the significance of the region immediately proximal to the X box (−127 to −114 bp) in terms of the enhancer activity. The −114/−66 sequence that included all of X and Y box sequences showed lower activity by about a factor of 6 than that of −160/−66 sequence, whereas deletion up to −128 bp did not result in reduced activity (when inserted in the natural orientation). DNase I footprinting (19) has already shown that the region protected by nuclear factor(s) in Raji cells extends at least 3 or 4 bp upstream of the 5′ end of the X box sequence, although protection further upstream than this could not clearly be seen because of apparent resistance of the adenosine tract (−127 to −118 bp) to DNase I digestion (see Fig. 1*A*). Moreover, additional sequence homologies among class II α and β chain genes in the region corresponding to −121 to −114 bp in the DQB sequence have also been noted (44). Therefore, it seems likely that the X box sequence involved in the interaction with transcriptional factor(s) extends from about −121 to −100 bp.

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