Cooperativity between the J and S Elements of Class II Major Histocompatibility Complex Genes As Enhancers in Normal and Class II–negative Patient and Mutant B Cell Lines

By Minoru Sugawara, Thomas Scholl, Sanjeev K. Mahanta, Paul D. Ponath, and Jack L. Strominger

From the Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138

Summary

The class II major histocompatibility complex genes all contain in their proximal promoters three *cis*-elements called S, X, and Y that are conserved in both sequence and position, and a fourth element, J, conserved in sequence but not in position. J, X, and Y and, to some extent, S, have been shown to be functionally important in regulation of expression of these genes. In the present study, a protein factor that binds cooperatively to the S plus J elements of the promoter of the class II major histocompatibility complex gene DPA has been detected. Moreover, functional cooperativity between S and J in activation of the enhancerless −40 interferon-β (−40 IFN-β) promoter has been demonstrated. Finally, the latter assay appears to subdivide complementation group A of class II negative human B cell lines that includes both mutants generated in vitro and cells from patients with the bare lymphocyte syndrome (type II). In three of these cell lines, the enhancerless −40 IFN-β promoter containing the S plus J elements was functionally active, while in the others it was inactive.

The human class II MHC genes play an essential role in class II MHC–restricted presentation of peptide antigens for generation of an antibody response and in some cases also for a class II MHC-restricted CTL response (Th1 cells) (1, 2). The regulation of class II protein cell surface expression is, therefore, an important first step in control of the initiation, character, and magnitude of an immune response. Inappropriate expression may lead to autoimmune disease, or alternatively, decreased expression of class II may enable evasion of recognition by T cells.

Moreover, a disorder of class II MHC gene regulation, the class II bare lymphocyte syndrome (BLS),[1] has been described (3–5). The disease is manifested by agammaglobulinemia, malabsorption, and T cell unresponsiveness to antigens and mitogens. The cell lines established from BLS patient's PBL as well as several similar mutant B cell lines generated in vitro do not express any of the three subtypes of class II antigens (HLA-DR, -DQ, and -DP) (6, 7), with the exception of one unusual cell line that expresses only DQ (8). Southern blots have shown that all of the α and β chain genes encoding these glycoproteins in a subregion of the human MHC on chromosome 6 are intact in these mutant cell lines, but Northern blots revealed that they have little or no class II mRNA. Somatic cell hybridization of each of these mutant cell lines with normal human or mouse B cell lines results in stable hybrid cell lines which express the class II genes of the mutant cells (9–12). The defect, which behaves as an autosomal recessive trait, does not segregate with the MHC, suggesting that the syndrome is due to a defective or inactive *trans*-acting transcription factor (TAF) (3, 13). Analysis of transient heterokaryons produced by fusion of all available mutant cell lines in vitro showed that they fall into at least three and probably four different complementation groups (14–17). The class II MHC–negative mutant cell lines provide an important system for identifying genes controlling class II MHC gene expression. Recently, a gene for a factor, CIITA, defective in RJ2.2.5 cells, a member of complementation group A, was isolated by complementation cloning (18, 19). Transfection of this gene restored expression of all class II MHC isotypes in RJ2.2.5 cells. The factor, however, is not a DNA binding protein but may represent a coactivator of the X and Y boxes.

A set of conserved upstream sequence elements, termed S, X, and Y boxes, are found in the proximal promoters of all class II MHC genes. Functional roles for the X and Y boxes in the regulation of class II gene expression has been

[1] *Abbreviations used in this paper:* BLS, bare lymphocyte syndrome; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay; hGH, human growth hormone; pRSV, plasmid Rous sarcoma virus; TAF, *trans*-acting transcription factor.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/07/0175/10 $2.00
Volume 182 July 1995 175–184

demonstrated by mutational analysis using 5', 3', and gap deletion as well as site-directed and linker scanning mutagenesis, and a number of factors that bind to these elements have been described (6, 7). The function of the S element (20) is not well understood although it is thought to participate in MHC class II gene regulation in a position-dependent manner (21–23). However, constructs containing the X and Y elements alone are inactive in B cells and are not restored by the S element itself (24). Moreover, a fourth element, J, that does restore activity of these constructs has been described that is conserved in sequence but not in position (24–26). In the present study both nuclear protein binding to and functional enhancer activities of the S and J elements of the HLA-DPA gene have been examined. Cooperative interaction between the S and the J elements of DPA is an important feature in both assays. Moreover, these studies appear to subdivide complementation group A of class II–negative B cell lines.

Materials and Methods

Reagents. rIFN-γ (referred to throughout as IFN-γ) was generously provided by Biogen (Cambridge, MA). The human growth hormone (hGH) assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA) and oligonucleotides (Oligos etc. Inc., Guilford, CT) were purchased.

Cell Lines. Raji (ATCC CCL86) is a Burkitt's lymphoma B cell line that expresses high levels of all three class II isotypes of proteins. HeLa (CCL2; American Type Culture Collection, Rockville, MD) is a cervical carcinoma cell line. While resting HeLa cells do not express class II determinants, HLA-DR and -DP, but not -DQ, can be induced by IFN-γ (27). HPB-ALL is a human T cell tumor line that does not express class II proteins.

The Epstein-Barr virus–transformed B cell lines derived from class II–deficient patients have been described elsewhere (16); BCH, BLS-2, Ramia, Nacera, and TF, and the laboratory-generated mutants, RJ2.2.5, RM-2, RM-3, Clone 13, and 6.1.6, were used and grown as previously. All except Clone 13 lack DR, DQ, and DP. Clone 13, however, express DQ selectively, but not DR or DP (8).

Plasmids and Assays. The control plasmid pRSVCAT (Rous sarcoma virus long-terminal repeat linked to the chloramphenicol acetyltransferase [CAT] gene), pTK CAT (TK promoter nucleotides −105 to +11 in pUC18 vector), and p40 IFN-β CAT (IFN-β promoter nucleotides −40 to +20 in pSP73 vector) have been described (28–30) and were the kind gift of T. Maniatis (Harvard University, Cambridge, MA). Oligonucleotides corresponding to the DPA and DQB sequences from positions −124 to −118 (S of DPA, 7 bp), −107 to −98 (J of DPA, 10 bp), −124 to −98 (S and J of DPA, 27 bp), −146 to −131 (J and S [or W] of DQB, 16 bp), were synthesized. Fragments corresponding to the minimum promoter region from positions −148 to −11 (DPA) or −160 to +39 (DQB) were purified after digestion with BamHI of the pDPA/148hGH plasmid and with HindIII of the pDQB/160CAT plasmid, respectively (24). These oligonucleotides and fragments were cloned into the BamHI site of the pUC18-CAT vector upstream of the TK promoter or into the BamHI site of the pSP73-CAT vector upstream of the −40 IFN-β promoter. Mutations in the S element, the J element, and the interspace region in the constructs containing the fragment −148 to −11 of the DPA promoter, and the S+J region of the DPA promoter in the −40 IFN-β CAT plasmid were made by PCR using the method of Giebel and Spritz (31). The 5' and the 3' primers corresponding to the sequences in the vector were 5'-TAATACGACTCACTATAGGG-3' and 5'-TTTTCTCCATTTTAGCTTCCTTAGCTCCTG-3', respectively. The primers used for making mutations in the S element, the interspace region, and the J element in the −148 to −11 DPA promoter fragment containing plasmid were 5'-CATCTCTTCCCCAGCA**TGCA**CCAGCGTCCTCTTTAC-3' 5'-CCCCAGCACCTTCCAG**ATCT**CTCTTTACCCAGCAAC-3' and 5'-GCGTCCTCTTT**T**CCCAGCAACAG-3', respectively. The primers used for making mutations in the S element, the interspace region, and the J element in the DPA S+J region containing plasmid were 5'-TCTAGAGGATCCAGCA**TGCA**CCAGCGTCCTCTTTAC-3', 5'-ATCCAGCACCTTCCAG**ATCT**CTCTTTACCCAGGGAT-3' and 5'-AGCGTCCTCTTT**T**CCCAGGGATCCC-3', respectively. The bold letters indicate the mutated bases.

Transfection of HeLa cell lines was performed by the calcium phosphate method as described (25, 32). Raji cells were transfected by electroporation in PBS plus 10 mM Hepes, pH 7.4 (24). Transfections also included 2 μg of pRSVhGH (33) as internal controls to normalize for small differences in transfection efficiencies (<15%). Approximately 48 h after transfection, the supernatant and cells were harvested by centrifugation and assayed for hGH activity and CAT activity essentially as described (33). For quantitative analysis a blot analyzer (Betascope 603; Betagen, Waltham, MA) was used (34).

Nuclear Extracts and Electrophoretic Mobility Shift Assay (EMSA). Nuclear extracts were prepared (35), from Raji, or confluent cultures of IFN-γ–treated and untreated HeLa cells using $\sim 10^8$ cells. Protein concentration was measured using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). The sequence of oligonucleotides used in the gel shift (only sense strand shown) experiments are 5'-GATCCC*GCACCTT*G, S element of DPA; 5'-GATCC**CTTTACCCAG**G-3', J element of DPA; 5'-GATCC*GCACCTT*CCAGCGTCCT**CTTTACCCAG**G-3', S and J elements of DPA; and 5'-GATCC**CTGGATTCAG***AACCTT*G-3' J and S elements of DQB. The sequences of the mutant oligonucleotides used in gel shift are as follows: 5'-GATCC*TACAAGG*CCAGCGTCCT**CTTTACCCAG**G-3', S+J of DPA with mutations in S; 5'-GATCC*GCACCTT*CCAGCGTCCT**AGGGCAAACT**G-3', S+J of DPA with mutations in J. The S element is italicized and underlined; the J element is in bold type and underlined. Each oligonucleotide has 5' and 3' BamHI linkers for cloning. The BamHI fragment of pDPA/148 (positions −148 to −11 of the DPA promoter), and EcoRI/HindIII fragment (51 bp) of pUC18 polylinker were also used. Gel shift assays were performed as described (36) with slight modification (24) using end-labeled DNA fragments.

Results

Protein Complexes Detected by EMSA Using Various J Element Probes. First, nuclear proteins were detected by EMSA with the J element probe of the DPA promoter (10 bp) alone using nuclear extracts of four different types of cells: Raji, which expresses class II MHC protein constitutively, HeLa, which is class II MHC protein negative, HeLa treated with IFN-γ to induce class II MHC protein expression, and HPB-ALL, a class II–negative human T cell line as a control. A single specific protein or protein complex that bound only weakly to the J element probe alone was detected in the class II–positive cell lines, Raji (Fig. 1 *A*, lane *2*) and IFN-γ induced HeLa (Fig. 1 *A*, lane *4*), but not in the two class II–nega-

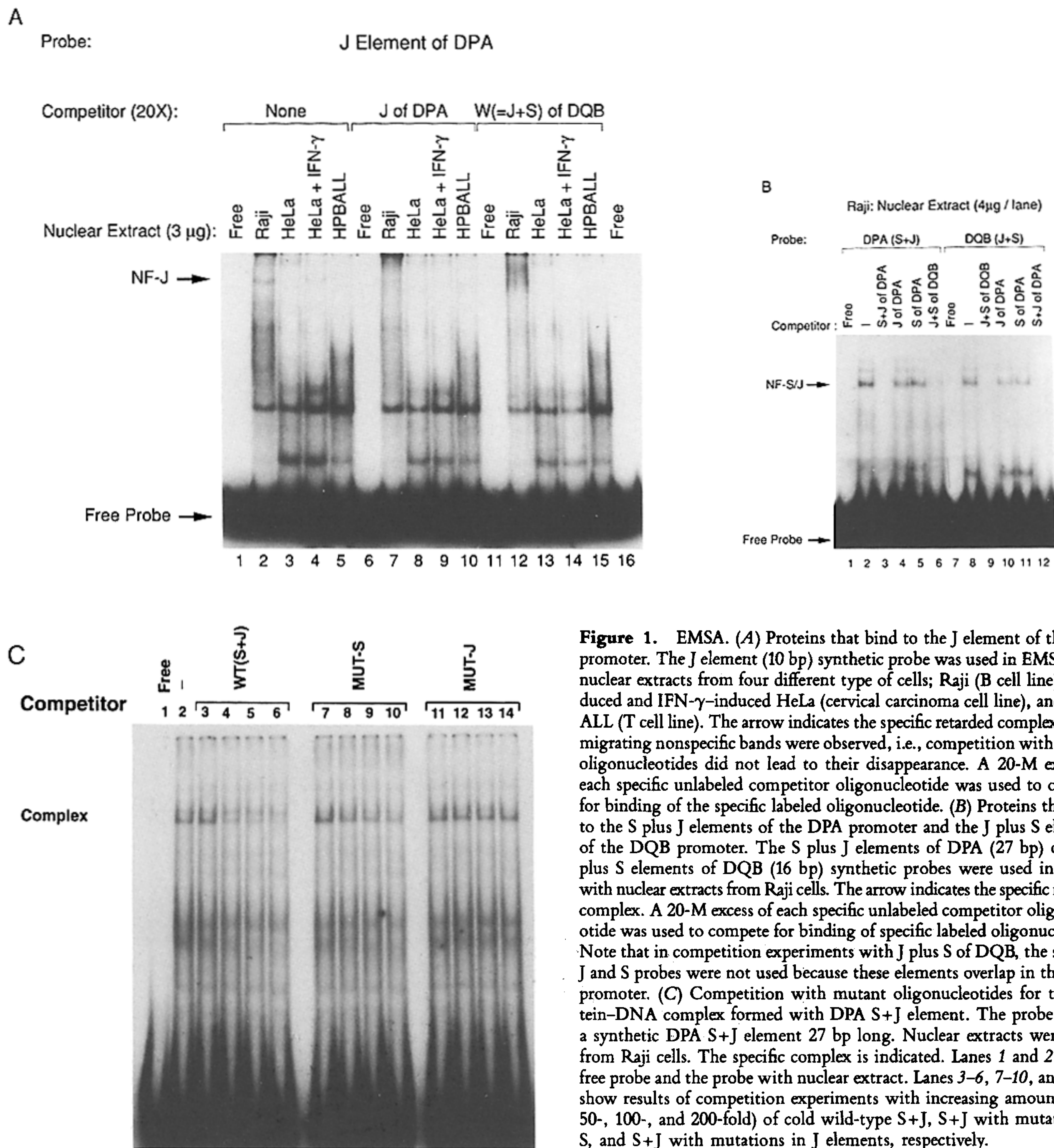


Figure 1. EMSA. (*A*) Proteins that bind to the J element of the DPA promoter. The J element (10 bp) synthetic probe was used in EMSA with nuclear extracts from four different type of cells; Raji (B cell line), uninduced and IFN-γ–induced HeLa (cervical carcinoma cell line), and HPB-ALL (T cell line). The arrow indicates the specific retarded complex. Faster migrating nonspecific bands were observed, i.e., competition with specific oligonucleotides did not lead to their disappearance. A 20-M excess of each specific unlabeled competitor oligonucleotide was used to compete for binding of the specific labeled oligonucleotide. (*B*) Proteins that bind to the S plus J elements of the DPA promoter and the J plus S elements of the DQB promoter. The S plus J elements of DPA (27 bp) or the J plus S elements of DQB (16 bp) synthetic probes were used in EMSA with nuclear extracts from Raji cells. The arrow indicates the specific retarded complex. A 20-M excess of each specific unlabeled competitor oligonucleotide was used to compete for binding of specific labeled oligonucleotide. Note that in competition experiments with J plus S of DQB, the separate J and S probes were not used because these elements overlap in the DQB promoter. (*C*) Competition with mutant oligonucleotides for the protein–DNA complex formed with DPA S+J element. The probe used is a synthetic DPA S+J element 27 bp long. Nuclear extracts were made from Raji cells. The specific complex is indicated. Lanes *1* and *2* are the free probe and the probe with nuclear extract. Lanes *3–6*, *7–10*, and *11–15* show results of competition experiments with increasing amounts (20-, 50-, 100-, and 200-fold) of cold wild-type S+J, S+J with mutations in S, and S+J with mutations in J elements, respectively.

tive cell lines (Fig. 1 *A*, lanes *3* and *5*). This complex was competed out by unlabeled synthetic J element probe (Fig. 1 *A*, lanes 7 and *9*) or by a synthetic probe that contained the overlapping J and S elements of DQB (Fig. 1 *A*, lanes *12* and *14*). Several nonspecific complexes were also observed that were not competed out by either of these probes. No specific protein bands could be observed using a synthetic S element of DPA probe (7 bp) (data not shown). These results strongly suggest that the J elements of DPA and DQB bind a factor(s) in common. Although the J element of DPA overlaps the 5′ end of the X box (X_1 box), that of DQB does not (24–26). Nevertheless, the possibility that the observed specific complex includes proteins that bind to X_1 is not excluded because some complexes may bind to several different elements and be linked by a common protein (see, for example, reference 37).

When synthetic probes encompassing both the S and the J elements of DPA (27 bp) or the J and S elements of DQB

(16 bp) (see Materials and Methods, and reference 24) were used as probes, a single band was again detected but had much greater intensity (Fig. 1 *B*). Strikingly, this band could not be competed out by synthetic J or S elements alone (Fig. 1 *B*, 20-fold molar excess, lanes *4*, *5*, *10*, and *11*), but only by the synthetic oligonucleotide containing both elements, derived either from DPA or from DQB (Fig. 1 *B*, lanes *3* and *9*). These data suggest that a cooperative interaction between the J and S elements occurs in the binding of this protein or protein complex, even though no binding to the S element alone could be detected. Moreover, the binding to the probe containing both elements (Fig. 1 *B*) was substantially stronger than that observed with the J element alone (Fig. 1 *A*). The results suggest that S and J elements of DPA and DQB bind a common factor and is compatible with an earlier report that the J element of DQB can function as a positive regulatory element for cooperation with the X and Y boxes of DPA (24). When the DPA S+J element was used as a probe and competition was carried out with unlabeled wild-type S+J element (Fig. 1 *C*, lanes *3–6*), or with S+J element with mutations in the S element (lanes *7–10*), or in the J element (see Materials and Methods) the wild-type S+J element competed very effectively. However, mutations in the S element alone reduced the ability of the oligonucleotide to compete with the wild-type probe for complex formation, while mutations in the J element abolished its ability to compete, thereby providing support for the suggestion that cooperativity exists between S and J element in the DPA promoter in the binding of nuclear proteins.

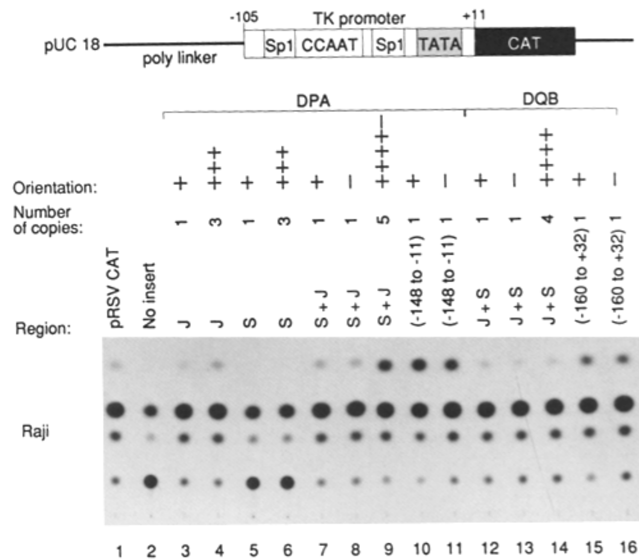


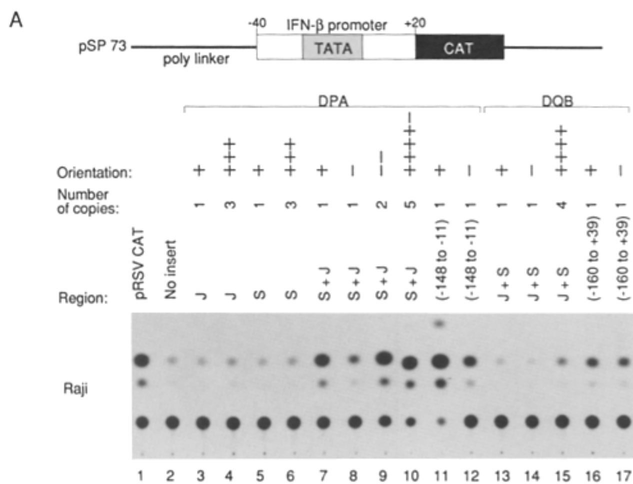
Figure 2. Plasmids containing DPA and DQB synthetic oligonucleotides linked to the TK promoter and their CAT activities in Raji B cells. 10 μg of CAT reporter plasmid and 2 μg of pRSVhGH normalization plasmid were transfected into Raji cells by electroporation as described in Materials and Methods. The SEM for the normalization plasmid was <15% in this and all following experiments. Crude extracts were assayed for CAT activity by TLC. This experiment was repeated three times in Raji cells with similar results each time. An autoradiogram of a typical assay is shown.

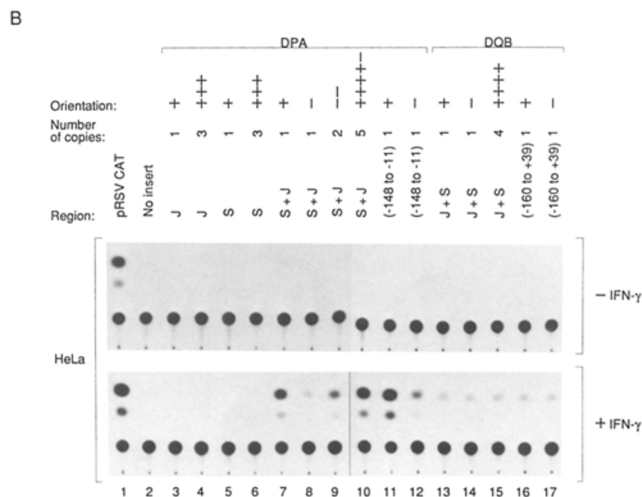
Enhancer Activity of the S and J Elements in CAT Assays. The role of the S and J elements as functional enhancers was examined using heterologous promoters. Two enhancerless promoters, the minimal TK promoter (which has three *cis*-elements, Sp1, CCAAT, and a TATA box) (29) and the −40 IFN-β promoter (which has only one *cis*-acting element, the TATA box) (30) have been used to examine the enhancer activity of the J and S elements alone or in combination. Using TK promoter, substantial activity of the enhancerless promoter alone was observed in the class II protein–positive B cell line, Raji (Fig. 2; compare lane *2*, no insert, with lane *1*, the positive control pRSVCAT). An increase in activity was observed with the entire DPA promoter (−148 to −11 bp) in either orientation (Fig. 2, lanes *10* and *11*). A lesser degree of enhancement was observed with the synthetic J element alone (Fig. 2, lanes *3* and *4*) and with the S plus J elements (Fig. 2, lanes 7 and *8*). A multimerized S plus J element gave maximal enhancement (Fig. 2, lane *5*). No enhancement was observed with the S element alone, either as a single copy or multimerized (Fig. 2, lanes *5* and *6*). Much weaker enhancement was observed with the DQB promoter in either orientation (Fig. 2, lanes *15* and *16*) or with its J plus S element (Fig. 2, lanes *12*, *13*, and *14*).

The enhancerless −40 IFN-β promoter yielded striking data. This promoter was virtually inactive in Raji cells (compare lanes *1* and *2* in Fig. 3 *A*) and similarly, the J and S elements alone of DPA were inactive either in single copy or multimerized form (Fig. 3 *A*, lanes *3–6*). However, the synthetic probe containing the S plus J elements in either orientation (Fig. 3 *A*, lanes 7 and *8*) and in a single copy as well as multimerized (Fig. 3 *A*, lanes 7, *9*, and *10*) were highly active relative to the intact promoter DPA (Fig. 3 *A*, lanes *11* and *12*). These data confirm the in vitro EMSA results (Fig. 1 *B*) in showing a cooperative interaction between the S and J elements using in vivo CAT activity. The DQB promoter or its J plus S elements were also active, but less so (Fig. 3 *A*, lanes *13–17*).

Similar results with the −40 IFN-β promoter were also obtained using HeLa cells (Fig. 3 *B*). No activity of any construct was demonstrable in uninduced HeLa cells. In IFN-γ–induced HeLa cells, the combination of S plus J elements of DPA was strikingly active as compared to either element alone (Fig. 3 *B*, lanes *3–6*), and was active in either orientation and as a monomer or multimer (Fig. 3 *B*, lanes *7–10*). The J plus S elements of DQB had minimal activity in this system corresponding to the fact that DQA and DQB genes cannot be induced by treatment with IFN-γ in HeLa cells (Fig. 3 *B*, lanes *13–17*).

The effect of mutations in the S element, in the interspace region and in the J element of the −40 IFN-β CAT plasmid containing either the −148 to −11 DPA promoter fragment or the S+J region of the DPA promoter alone are shown in Fig. 3 *C*. Mutations in either the S element or the J element alone significantly reduced the activity as they did in the gel shift assay (Fig. 1 *C*). The effect of mutation in the J element was again greater than the effect of mutation in





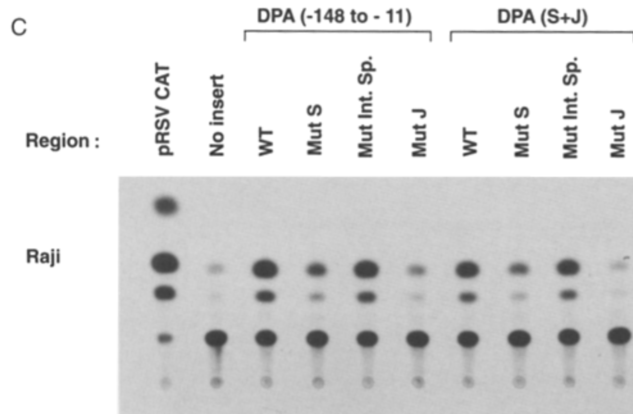


Figure 3. Plasmids containing DPA and DQB synthetic oligonucleotides linked to the −40 IFN-β promoter and their CAT activities in Raji, and uninduced and IFN-γ–induced HeLa cells. (*A*) In Raji cells. 10 μg of CAT reporter plasmid and 2 μg of pRSVhGH normalization plasmid were transfected into Raji cells by electroporation as described in Materials and Methods. Crude extracts were assayed for CAT activity by thin layer chromatography. This experiment was repeated three times in Raji cells with similar results each time. An autoradiogram of a typical assay is shown. (*B*) In HeLa cells without or with IFN-γ treatment. 10 μg of CAT reporter plasmid and 2 μg of pRSVhGH normalization plasmid were transfected into HeLa cells by the calcium phosphate method as described in Materials and Methods. 2 d after treating the transfectants with IFN-γ (500 U/ml), crude extracts were assayed for CAT activity by TLC. These experiments were repeated three times in HeLa cells with similar results each time. An autoradiogram of a typical assay is shown. (*C*) 10 μg of CAT reporter plasmid was transfected into Raji cells by electroporation as described in Materials and Methods. Crude extracts were assayed for CAT activity by thin layer chromatography and subsequent quantitation on a Betascope. CAT activities are reported as relative CAT activity where the activity of the wild-type insert is considered as 100%. Mutations in the S element, the interspace region, and the J element in the −148 to −11 DPA promoter construct brought down the CAT activity to 19, 87, and 7% of the wild-type, respectively. Mutations in the S element, the interspace region, and the J element in the context of DPA S+J brought down the activity to 19, 80, and 6% of the wild type, respectively. The CAT activities reported are the average of three independent transfections. The actual percentage of conversion for the wild-type insert is shown in parentheses. Each number represents the arithmetic mean of three independent transfections. The SEM was <20%.

the S element. Mutations in the interspace region had little or no effect.

Transfection of the −40 IFN-β Promoter CAT Constructs into Normal and Class II Protein–negative Mutant BLS Patient Cell Lines. Examination of B cell lines belonging to various BLS complementation groups in comparison to Raji cells as well as to HeLa cells (without or with induction by IFN-γ) was next carried out (Table 1 and Fig. 4) to determine if any of the transactivating defects in these cell lines is linked to the S and J elements. For these experiments, the intact DPA promoter (pDPA/148) which could activate the −40 IFN-β promoter in Raji cells and IFN-γ induced HeLa (Fig. 3 *A*) was used as a control. As expected, this promoter had nearly basal activity in all of the class II–negative cell lines. Similarly to Raji cells, in cells of complementation groups B, C, D, and in three of the six cells in group A, the S plus J elements together activated the −40 IFN-β promoter, but no activity was observed with either element alone (Table 2 and Fig. 4). By contrast, in the other three cell lines in complementation group A, RM-2, Clone13, and BCH, no activity could be detected. Clone 13 and BCH had already been recognized to differ in some respects from other members of complementation group A (16, 38), and these experiments appear to subdivide this complementation group further.

Discussion

The control of gene regulation requires protein–protein and protein–DNA interaction. Many transcription factors mediate the regulation of DNA transcription by virtue of their ability to specifically interact with particular DNA sequences and proteins of the basal transcription apparatus (39–42). These transcription factors contain at least two domains: the DNA binding region which positions the protein on the DNA and the activating region that interacts with the transcription complexes (43). These transcription com-

Table 1. *Activities of −40 IFN-β CAT Reporter Plasmids in Different Mutants of Complementation Group A*

Cell lines	Relative CAT activity of −40 IFN-β promoter plasmids (%)					
	pRSVCAT	−	J	S	S + J	pDPA/148
Controls	%	%	%	%	%	%
Raji	100 (45.4)	6.7	6.6	5.8	76	207
HeLa (−IFN-γ)	100 (38.3)	3.1	3.0	3.7	2.6	3.6
(+IFN-γ)	100 (34.4)	7.8	4.8	5.1	38	135
BLS phenotype						
Complementation group A (type II)						
BCH	100 (16.5)	0.5	0.4	0.4	1.5	1.0
BLS-2	100 (12.9)	0.7	0.6	0.6	44	17
RJ2.2.5	100 (66.1)	2.3	1.8	2.6	64	14
RM-2	100 (56.0)	2.5	1.7	1.8	9.8	5.7
RM-3	100 (52.1)	2.8	1.8	2.0	23	3.4
Clone 13	100 (93.3)	1.3	1.0	1.1	4.4	7.1
Complementation group B (type III)						
Nacera	100 (36.2)	1.0	0.9	0.9	77	7.9
Ramia	100 (49.8)	0.8	0.9	0.7	15	1.5
Complementation group C (type III)						
TF	100 (37.1)	0.5	0.8	0.6	16	1.0
Complementation group D (type ?)						
6.1.6	100 (21.3)	5.3	5.0	5.0	58	21

10 μg of CAT reporter plasmid and 2 μg of pRSVhGH normalization plasmid were transfected into Raji, uninduced, and IFN-γ–induced HeLa and class II–negative mutant and BLS patient cells by electrophoration as described in Materials and Methods. Crude extracts were assayed for CAT activity by TLC and subsequent quantitation on a Betascope. CAT activities are reported as relative CAT activity standardized to pRSVCAT (100%) and are the average results of three to four independent transfections. The actual percentage of chloramphenicol acetylated using pRSVCAT in this series of transfections is shown in parentheses in the first column. Each number represents the arithmetic mean of at least three independent transfections. The SEM was $<20\%$.

plexes include proteins (coactivators) that do not bind to DNA but mediate interactions between the DNA binding proteins. Regulation of transcription can be accomplished by modulating the quantities or activities of sequence-specific transcription factors. "Basal-level" transcription from RNA polymerase II promoters involves the formation of a multiprotein complex at the TATA box region, which contains RNA polymerase II and a set of "general" transcription factors, such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH. "Activated" transcription requires the binding of "regulatory" transcription factors to other DNA sequences in the promoter. These regulatory factors are thought to mediate activation by forming protein–protein interactions with the general transcription factors, sometimes through the protein–protein interactions of coactivators (44). Direct coordination between the regulatory gene specific or tissue specific and general transcription factors are not yet well established. However, four viral transactivating proteins, the adenovirus E1A (45, 46), the herpes virus VP16 (47), the Epstein-Barr virus Zta (48), and the cytomegalovirus IE2 (49), have been shown to contact directly the TATA box binding protein TFIID and, in the case of VP16, the general factor TFIIB (50).

The class II X and Y boxes are regulatory transcription elements necessary but not sufficient for maximal transcription of class II MHC genes. These *cis*-elements have been shown to bind the transcription factors RF-X (X_1 box), hXBP-1 (X_2 box), and NF-Y_A and Y_B (Y box) (6, 7). Other upstream elements such as the pyrimidine tract (Py), the S (also included in or called W, Z, or H), and the J element are required in addition to the X and Y boxes (6, 7, 24–26).

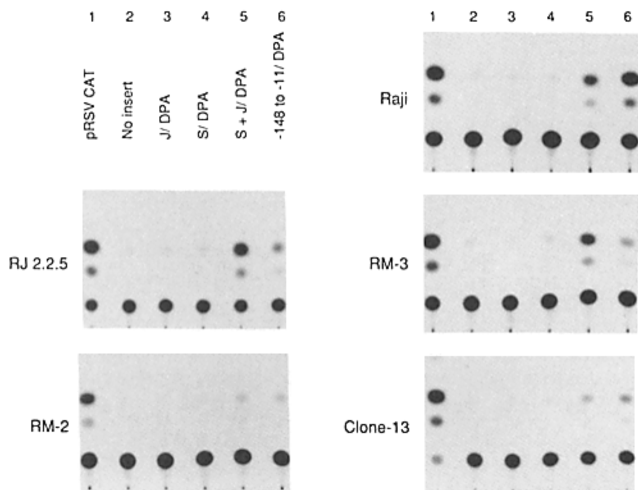


Figure 4. Activities of −40 IFN-β CAT reporter plasmids in different members of complementation group A. The control plasmids (pRSVCAT) and −40 IFN-β plasmids used (Fig. 3 *A*) are shown in the upper left box. All of those inserts derived from the DPA gene had a single copy in the sense (+) orientation. Raji, the control cell line, is shown in the upper right box. Different subgroups of complementation group A (see Table 2) are illustrated in the remaining boxes.

The W box was shown to be important for optimal levels of B cell expression and essential for IFN-γ induction (51–53). Two protein complexes were preliminarily shown to interact with the W box of the DRA gene, which includes overlapping J and S elements (54, 55). In a previous paper, the possible role of the S and J elements in transcription of human class II MHC genes was examined. The S element is a conserved sequence element found 15 bp upstream of the X and Y elements. X and Y function together as a specific enhancer of class II MHC gene expression (33). S element has been shown to be functionally important for the DRA promoter (21, 22). Later, as the results of 5′ deletion and mutagenesis analysis, a fourth element, called the J element, was found in the DPA and DQB genes (24). A similar sequence element was located in the proximal promoters of each of the other human and murine class II MHC genes, but its possible functional role in these other genes has not been examined. The J element is located at different positions in the DPA, DQB, and other class II MHC genes and thus had not previously been detected by sequence alignments (24–26). Recently, two DNA binding proteins that bind to the J element, but only in constructs which also contain the S element, were identified. One of these, encoded by clone 18, has been studied in detail (56). This protein contains a novel conserved domain at its NH_2 terminus, a central acidic activation region, and a COOH-terminal DNA binding region containing 11 zinc fingers. Antisense constructs of each these new J element binding proteins dramatically inhibited IFN-γ–induced class II MHC gene expression in HeLa cells, but constitutive expression in established B cell lines was more difficult to inhibit (Sugawara, M. unpublished observations).

The present paper provides additional evidence that the S and J elements function together in the transcription of class II MHC genes. First, in extracts of the Raji B cell line, a DNA binding complex was detected with the J element probe alone (Fig. 1 *A*), but was much more prominent using probes containing both the S and J elements (Fig. 1 *B*). Similarly, in competition experiments with oligonucleotides, competition could be observed only with competitors containing both S and J (Fig. 1 *B*). Also, mutations in the DPA promoter S and J elements individually, but in the context of the S plus J region, led to a significant loss in the ability of the mutant oligonucleotides to compete with the wild-type probe for the complex (Fig. 1 *C*). Finally, using two enhancerless promoters, the TK promoter (Fig. 2) and the −40 IFN-β promoter (Fig. 3), the cooperativity of the S and J elements as enhancers, both in Raji cells which constitutively express class II MHC protein and in IFN-γ–induced HeLa cells, was dramatically illustrated. Two different S and J element sequences were used, that from DPA gene and that from the DQB gene, in all of these experiments. The two elements have different relative locations in these two genes. In the DPA gene, the J element is located 10 bp downstream from the S element, and overlaps the X_1 box. In the DQB gene, the J element is located immediately upstream of the S element, which is overlapped by 1 bp. In the DPA gene in particular, cooperativity of the two elements separated by 10 bp is unusual. In any event, these results strongly suggest that the cooperativity of the S plus J elements is a major transcriptional feature of regulation in class II antigen–positive Raji and IFN-γ–treated HeLa cells. Further supportive evidence (Fig. 3 *C*) was provided by the −40 IFN-β promoter CAT assays which showed that mutations made in the S or the J element alone in the context of either the −148 to −11 DPA promoter fragment or the DPA S+J region led to significant loss in CAT activity. Mutations made in the interspace region had a minor influence.

Finally, the function of the S plus J enhancer in class II–negative human B cell lines obtained either from patients with the BLS or produced by in vitro mutagenesis has been examined (Fig. 4 and Table 2). These lines have previously been shown to fall into three and possibly four complementation groups. Members of each group have been examined. As expected, the full proximal promoter of the DPA gene (−148 to −11 bp containing the S, J, X, and Y elements) when

Table 2. *Phenotype of Cells of Complementation Group A*

Cell lines	Activity of S + J*	DQ Expression‡
Raji (control)	+	+
RJ2.2.5, RM-3, BLS-2	+	−
Clone 13	−	+
RM-2, BCH	−	−

* Enhancer activity using the −40 IFN-β heterologous promoter.
‡ Surface expression using the mAb Leu 10 for FACS® analysis.

cloned upstream of the −40 IFN-β promoter was inactive in all of the class II–negative cell lines, although high level activity of the same constructs was observed in Raji cells and in IFN-γ–induced HeLa cells. However, the S plus J elements cloned upstream of the −40 IFN-β promoter were fully active in cells of complementation groups B, C, and D, indicating that the factor missing or defective in these groups does not involve these elements. With cells representing complementation group A, a surprising anomaly was found. In three members of this group (RJ2.2.5, RM-3, and BLS-2), the S plus J elements were highly active, despite the lack of activity of the intact DPA promoter, as in groups B, C, and D. By contrast, in clone 13, RM-2, and BCH, the activity was either very low or absent (57). Thus, these *cis*-acting S and J elements must somehow be involved as a target(s) of transcription factor(s) missing, defective, or inactive in the RM-2, clone 13, and BCH mutant B cell lines. Moreover, this assay subdivided members of complementation group A further. Previously, clone 13 had been distinguished from other members of group A by the fact that it expresses normal levels of DQ, although it is negative for DR and DP, while other members of the group, including both RM-2 and BCH, are negative for all three isotypes of class II protein. Thus, these experiments define three subgroups of complementation group A (Table 2). In addition, BCH has previously been shown to differ from other members of the subgroup by in vivo footprinting (38). A genetic defect has been identified in two members of group A cells, the in vitro mutant cell line RJ2.2.5 and the patient cell line BLS-2. This factor CIITA, which is IFN-γ inducible, and is not a DNA binding protein, was shown to have a large deletion in RJ2.2.5 and to be missing an exon of 24 amino acids in the mRNA in BLS-2, as the consequence of a mutation in a splice donor site (18). The other members of this complementation group have not yet been examined, and thus one possibility would be that they differ in the precise defect in CIITA. Since these defects do not complement each other, (i.e., they all belong to complementation group A), they must carry mutations in the same gene. One plausible interpretation would be that this gene/protein, i.e., CIITA, interacts with multiple transcription factors (i.e., factors that bind to J, S, X, Y, TATA, and/or YY1 [58] boxes), and that the different defects involve mutations in different regions of CIITA specific for these different interactions. For example, the defects in clone 13, RM-2, and BCH may involve a region required for interaction with S plus J element binding proteins and would be reflected in the inability of the S/J elements in the heterologous promoter construct, the -40 IFN-β promoter. (For an earlier discussion of the anomaly of clone 13, see reference 16.) In any event, the manner in which the putative genetic defects might relate to the failure of the S plus J elements to function as enhancers in some cells of this complementation group and their normal function in other mutants remain to be examined.

We thank Ms. Kahori Sugawara for excellent technical assistance.

This work was supported by National Institutes of Health grants DK30241 and CA47554 to J. L. Strominger. P. D. Ponath was the recipient of an Arthritis Investigator award, T. Scholl of an American Cancer Society fellowship, and S. K. Mahanta of an Irvington Institute fellowship.

Address correspondence to J. L. Strominger, Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138. M. Sugawara's current address is AGENE Research Institute, c/o Nippon Roche K.K. 200 Kajiwara, Kamakura City, Kanagawa, 247 Japan. P. D. Ponath's current address is LeukoSite, Inc., 215 1st Street, Cambridge, MA 02139.

Received for publication 25 August 1994 and in revised form 6 March 1995.

References

1. Unanue, E.R. 1984. Antigen-presenting function of macrophage. *Annu. Rev. Immunol.* 2:395–428.
2. Germain, R.N., and D.H. Margulies. 1993. The biochemistry and cell biology of antigen processing and presentation. *Annu. Rev. Immunol.* 111:403–450.
3. Lipowska-Grospierre, B., A. Durandy, J.-L. Virelizier, A. Fisher, and C. Griscelli. 1983. Combined immunodeficiency with defective expression of HLA: modulation of an abnormal HLA synthesis and functional studies. *Birth Defects Orig. Artic. Ser.* 19:87–92.
4. Hadam, M.R., R. Dopfer, G. Dammer, H.-H. Peter, M. Schlesier, C. Mueller, and D. Niethammer. 1984. Defective expression of HLA-D-region determinants in children with congenital agammaglobulinemia and malabsorption: a new syndrome. *In* Histocompatibility Testing. E.D. Albert, M.P. Baur, and W.R. Mayr, editors. Springer-Verlag, New York. pp. 645–650.
5. de Preval, C., B. Lisowka-Grospierre, M. Loche, C. Griscelli, and B. Mach. 1985. A *trans*-acting class II regulatory gene unlinked to the MHC controls expression of HLA class II genes. *Nature (Lond.).* 318:291–293.
6. Benoist, C., and D. Mathis. 1990. Regulation of major histocompatibility complex class II genes: X, Y and other letters of the alphabet. *Annu. Rev. Immunol.* 8:681–715.
7. Glimcher, L.H., and C. Kara. 1992. Sequences and factors: a guide to MHC class-II transcription. *Annu. Rev. Immunol.* 10:13–49.
8. Ono, S.J., V. Bazĭl, M. Sugawara, and J.L. Strominger. 1991. An isotype-specific *trans*-acting factor is defective in a mutant B cell line that expresses HLA-DQ, but not -DR or -DP. *J.*

Exp. Med. 173:629–637.
9. Gladstone, P., and D. Pious. 1980. Identification of a *trans*-acting function regulating HLA-DR expression in a DR-negative B cell variant. *Somatic Cell Genet.* 6:285–298.
10. Salter, R.D., J. Alexander, F. Levine, D. Pious, and P. Cresswell. 1985. Evidence for two *trans*-acting genes regulating HLA class II antigen expression. *J. Immunol.* 135:4235–4238.
11. Accolla, R.S., G. Carra, and J. Guardiola. 1985. Reactivation by a *trans*-acting factor of human major histocompatibility complex Ia gene expression in interspecies hybrids between an Ia-negative human B-cell variant and an Ia-positive mouse B-cell lymphoma. *Proc. Natl. Acad. Sci. USA.* 82:5145–5149.
12. Accolla, R.S., L. Scarpellino, G. Carra, and J. Guardiola. 1985. *Trans*-acting element(s) operating across species barriers positively regulate expression of major histocompatibility complex class II genes. *J. Exp. Med.* 162:1117–1133.
13. Accolla, R.S. 1983. Human B cell variants immunoselected against a single Ia antigen subset have lost expression of several Ia antigen subsets. *J. Exp. Med.* 157:1053–1058.
14. Yang, Z., R.S. Accolla, D. Pious, B.J.M. Zegers, and J.L. Strominger. 1988. Two distinct genetic loci regulating class II gene expression are defective in human mutant and patient cell lines. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1965–1972.
15. Hume, C.R., and J.S. Lee. 1989. Congenital immunodeficiencies associated with absence of HLA class II antigens on lymphocytes result from distinct mutations in *trans*-acting factors. *Hum. Immunol.* 26:288–309.
16. Benichou, B., and J.L. Strominger. 1991. Class II-antigen-negative patient and mutant B-cell lines represent at least three, and probably four, distinct genetic defects defined by complementation analysis. *Proc. Natl. Acad. Sci. USA.* 88:4285–4288.
17. Seidl, C., C. Saraiya, Z. Osterwell, Y.P. Fu, and J.S. Lee. 1992. Genetic complexity of regulatory mutants defective for HLA class II gene expression. *J. Immunol.* 148:1576–1584.
18. Steimle, V., L.A. Otten, M. Zufferey, and B. Mach. 1993. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or Bare Lymphocyte Syndrome). *Cell.* 75:135–146.
19. Steimle, V., C.-A. Siegrist, A. Mottet, B. Lisowska-Grospierre, and B. Mach. 1994. Regulation of MHC class II expression by interferon-γ mediated by the transactivator gene CIITA. *Science (Wash. DC).* 265:106–109.
20. Servenius, B., L. Rask, and P.A. Peterson. 1987. Class II genes of the human major histocompatibility complex. *J. Biol. Chem.* 262:8759–8766.
21. Vilen, B.J., J.F. Penta, and J.P.-Y. Ting. 1992. Structural constraints within a trimeric transcriptional regulatory region. *J. Biol. Chem.* 267:23728–23734.
22. Basta, P.V., P.A. Sherman, and J.P.-Y. Ting. 1988. Detailed delineation of an interferon-γ-responsive element in human HLA-DRA gene expression in a glioblastoma multiforma line. *Proc. Natl. Acad. Sci. USA.* 85:8618–8622.
23. Dedrick, R.L., and P.P. Jones. 1990. Sequence elements required for activity of major histocompatibility complex class II promoter bind common and cell type specific nuclear factors. *Mol. Cell. Biol.* 10:593–604.
24. Sugawara, M., P.D. Ponath, J. Shin, Z. Yang, and J.L. Strominger. 1991. Delineation of a previously unrecognized *cis*-acting element required for HLA class II gene expression. *Proc. Natl. Acad. Sci. USA.* 88:10347–10351.
25. Yang, Z., M. Sugawara, P.D. Ponath, L. Wessendorf, J. Banerji, Y. Li, and J.L. Strominger. 1990. Interferon γ response region in the promoter of the human DPA gene. *Proc. Natl. Acad. Sci. USA.* 87:9226–9230.
26. Sugawara, M., P.D. Ponath, Z. Yang, and J.L. Strominger. 1992. Interferon-γ response region in the promoter of the class II MHC gene, DPA. *Hum. Immunol.* 35:157–164.
27. Blanck, G., M. Lok, K. Lok, E. Downie, J.H. Korn, and J.L. Strominger. 1990. γ-Interferon induction of HLA class II mRNAs in dermal fibroblasts defined by RNAse protection analysis. *Hum. Immunol.* 29:150–156.
28. Gorman, C.M., G.T. Merlino, M.C. Willingham, I. Pastan, and B.H. Howard. 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. *Proc. Natl. Acad. Sci. USA.* 79:6777–6781.
29. Luckow, B., and G. Shutz. 1987. CAT constructions with multiple unique restriction site for the functional analysis of eukaryotic promoters and regulatory elements. *Nucleic Acids Res.* 15:5490.
30. Keller, A.D., and T. Maniatis. 1991. Identification and characterization of a novel repressor of β-interferon gene expression. *Genes & Dev.* 5:868–879.
31. Giebel, L.B., and R.A. Spritz. 1990. Site directed mutagenesis using a double stranded DNA fragment as a PCR primer. *Nucleic Acids Res.* 18:4947.
32. Chen, C., and H. Okayama. 1987. High-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.* 7:2745–2752.
33. Sakurai, M., and J.L. Strominger. 1987. B-cell-specific enhancer activity of conserved upstream elements of the class II major histocompatibility complex DQB gene. *Proc. Natl. Acad. Sci. USA.* 85:6909–6913.
34. Sullivan, D.E., P.E. Auron, G.J. Quigley, P. Watkins, J.E. Stanchfield, and C. Bolon. 1987. The nucleic acid blot analyzer. I. High speed imaging and quantitation of ^{32}P-labeled blots. *Biotechniques.* 5:672–678.
35. Digman, J.D., R.H. 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.
36. Singh, H., R. Sen, D. Baltimore, and P.A. Sharp. 1986. A nuclear factor that finds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. *Nature (Lond.).* 319:154–158.
37. Cogswell, J.P., P.V. Basta, and J.P.-Y. Ting. 1990. X-box-binding proteins positively and negatively regulate transcription of the HLA-DRA gene through interaction with discrete upstream W and V elements. *Proc. Natl. Acad. Sci. USA.* 87:7703–7707.
38. Kara, C.J., and L.H. Glimcher. 1993. Three in vivo promoter phenotypes in MHC class II deficient combined immunodeficiency. *Immunogenetics.* 37:227–230.
39. Berk, A.J., and M.C. Schmidt. 1990. How *do* transcription factors work? *Genes & Dev.* 4:151–155.
40. Johnson, P.F., and S.L. McKnight. 1989. Eukaryotic transcriptional regulatory proteins. *Annu. Rev. Biochem.* 58:799–839.
41. Mitchell, P.J., and R. Tjian. 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science (Wash. DC).* 245:371–378.
42. Ptashne, M., and A.F. Gann. 1990. Activators and targets. *Nature (Lond.).* 346:329–331.
43. Ptashne, M. 1988. How eukaryotic transcriptional activators work. *Nature (Lond.).* 335:683–689.
44. Greenblatt, J. 1991. Roles of TFIID in transcriptional initiator by RNA polymerase II. *Cell.* 66:1067–1070.

45. Lee, W.S., C.C. Kao, G.O. Bryant, X. Liu, and A.J. Berk. 1991. Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. *Cell.* 67:365–376.
46. Horikoshi, N., K. Maguire, A. Kralli, E. Maldonado, D. Reinberg, and R. Weinmann. 1991. Direct interaction between adenovirus E1A protein and the TATA box binding transcription factor IID. *Proc. Natl. Acad. Sci. USA.* 88:5124–5128.
47. Stringer, K.F., C.J. Ingles, and J. Greenblatt. 1990. Direct and selective binding of an acidic transcriptional activator domain to TATA-box factor TFIID. *Nature (Lond.).* 345:783–786.
48. Lieberman, P.M., and A.J. Berk. 1991. The Zta *trans*-activator protein stabilizes TFIID association with promoter DNA by direct protein-protein interaction. *Genes & Dev.* 5:2441–2454.
49. Hagemeier, C., S. Walker, R. Caswell, T. Kouzarides, and J. Sinclair. 1991. The human cytomegalovirus 80-Kilodalton but not the 72-Kilodalton immediate-early protein transactivates heterologous promoters in a TATA box-dependent mechanism and interacts directly with TFIID. *J. Virol.* 66:4452–4456.
50. Lin, Y.-S., I. Ha, E. Maldonado, D. Reinberg, and M.R. Green. 1991. Binding of general transcription factor TFIIB to an acidic activator region. *Nature (Lond.).* 353:569–571.
51. Boss, J.M., and J.L. Strominger. 1986. Regulation of a transfected human class II major histocompatibility complex gene in human fibroblasts. *Proc. Natl. Acad. Sci. USA.* 83:9139–9143.
52. Cogswell, J.P., J. Austin, and J.P.-Y. Ting. 1991. The W element is a positive regulator of HLA-DRA transcription in various DR+ cell types. *J. Immunol.* 146:1361–1367.
53. Tsang, S.Y., M. Nakanishi, and B.M. Peterlin. 1988. B-cell-specific and interferon-gamma-inducible regulation of the HLA-DR alpha gene. *Proc. Natl. Acad. Sci. USA.* 85:8598–8602.
54. Miwa, K., C. Doyle, and J.L. Strominger. 1987. Sequence-specific interactions of nuclear factors with conserved sequences of human class II major histocompatibility complex genes. *Proc. Natl. Acad. Sci. USA.* 84:4939–4943.
55. Tsang, S.Y., M. Nakanishi, and B.M. Peterlin. 1990. Mutational analysis of the DRA promoter: *cis*-acting sequences and *trans*-acting factors. *Mol. Cell. Biol.* 10:711–719.
56. Sugawara, M., T. Scholl, P.D. Ponath, and J.L. Strominger. 1994. A factor that regulates the class II major histocompatibility complex gene DPA is a member of a subfamily of zinc finger proteins that includes a *Drosophila* developmental control protein. *Mol. Cell. Biol.* 14:8438–8450.
57. Calman, A.F., and M.B. Peterlin. 1987. Mutant human B cell lines defective in class II major histocompatibility complex transcription. *J. Immunol.* 139:2489–2495.
58. Hehlgans, T., and J.L. Strominger. 1995. Activation of transcription by binding of NF-E1 (YY1) to a newly identified element in the first exon of the human DRα gene. *J. Immunol.* 139:2489–2495.