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Immunology

Interferon γ response region in the promoter of the human *DPA* gene

(promoter deletion/heterologous promoter/cis-acting elements/interferon-γ inducibility)

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ABSTRACT **The interferon γ (IFN-γ) response region of the human class II major histocompatibility complex gene, *DPA*, has been localized to a 52-base-pair (bp) DNA fragment in the proximal promotor at −107 to −55 bp after transfection into HeLa cells of a series of 5′, 3′, and gap deletion mutants linked to a reporter gene, human growth hormone, as well as of synthetic oligonucleotides fused to the heterologous promoter thymidine kinase. The 52-mer sequence contains the X and Y box elements conserved in all class II genes; their presence is indispensable for IFN-γ inducibility. Furthermore, an additional 5 bp immediately 5′ of the X box of the DPA gene are necessary and sufficient for IFN-γ induction. This region may contain an IFN-γ response element. A closely related sequence has also been found in the vicinity of the critical deletion sites of three other well-studied class II gene promoters, all of which require a much longer sequence 5′ of the X box. A fourth element, the W element, located about 15 bp 5′ of the X box in all class II genes, is clearly of little importance in IFN-γ inducibility of the *DPA* gene.**

The expression of the highly polymorphic class II major histocompatibility complex (MHC) molecules is essential for immunocompetent cells to present many foreign antigens to T cells in the generation of specific antibody responses (1). In humans, at least three functional class II molecules occur—namely DR, DQ, and DP. The genes encoding their α and β chains are located within a 1-megabase region of the MHC on chromosome 6 (2). Their constitutive expression is restricted to certain cell types—i.e., B cells, macrophages, some activated T cells, thymic epithelial cells, and dendritic cells. However, their expression can also be induced in a broad range of cell types by lymphokines, such as interferon γ (IFN-γ) and interleukin 4 (3–5). IFN-γ increases the steady-state levels of class II mRNA and the transcription rates of class II genes (6–8). Thus, the induction of class II gene expression is regulated primarily at the transcriptional level. Two highly conserved upstream sequences, termed the X and Y boxes, are contained within the 5′ flanking sequences of all class II genes (9, 10) and play a crucial role in the transcriptional regulation of these genes, functioning together as a B-cell-specific enhancer (11). The cis regulatory elements involved in IFN-γ induction have been intensively investigated in the murine *Eα* and human *DRA* and *DQB* promoters (12–15). However, an IFN-γ response element(s) (IRE) has not yet been clearly identified. In the present studies, an IFN-γ response region has been localized in the 5′ flanking sequences of the class II *DPA* gene by a series of 5′, 3′, and gap deletion mutants, as well as by heterologous promoter studies.

MATERIALS AND METHODS

Cell Line and Reagents. The HeLa cell line, derived from a human cervical carcinoma, was obtained from the American Type Culture Collection. It was maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum. The endogenous class II *DR* and *DP* genes in this line can be induced by IFN-γ, while *DQ* genes cannot be induced (16). Recombinant human IFN-γ (referred to throughout as IFN-γ) was generously provided by Biogen. SP6 polymerase (Promega), the hGH assay kit (Hybritech), and oligonucleotides (Research Genetics, Huntsville, AL) were purchased.

Plasmid Construction and BAL-31 Nuclease Deletion. The 2.3-kilobase *Bam*HI/*Hin*dIII DNA fragment (which separates the head-to-head *DPA* and *DPB* genes and therefore contains their promoters) from plasmid pDP3 (subcloned from cosmid G17A) (5, 17, 18) was subcloned into the *Bam*HI/*Hin*dIII site of pUC18. The plasmid pDPA/O was made by ligating a *Bam*HI/*Eco*RI fragment containing the hGH gene from p0GH into the pSP73 vector. pDPA/148 was constructed by subcloning a *Kpn* I/*Bam*HI fragment, which contains the *DPA* promoter from −148 to −11 base pairs (bp) into the *Bam*HI/*Kpn* I site of the polylinker of plasmid pDPA/O. The 3′ deletion series was generated by slow BAL-31 nuclease treatment at the *Bam*HI-cut pDPA/148. After BAL-31 treatment, *Bam*HI linkers were added to the ends of the DNA and the BAL-31-damaged hGH gene was replaced by an intact hGH gene from plasmid pDPA/O. The 5′ deletion series was made by a similar technique but the BAL-31 treatment started from the *Kpn* I site (−148 bp) of pDPA/148 and *Bgl* II linkers were added to the ends of BAL-31-treated DNA. All plasmids from either 5′ or 3′ deletions were size selected and then subjected to DNA sequencing to determine the precise deletion. The plasmid pDPA/148tk was constructed by three-part ligation of an *Xba* I/*Bam*HI fragment of the *DPA* promoter from position −148 to −11 bp, a *Bam*HI/*Hin*dIII fragment from ptkLS-115 containing a 115-bp thymidine kinase (tk) promoter and the entire tk gene (19, 20), and an *Xba* I/*Hin*dIII-digested Bluescript vector (Stratagene). Additional plasmids described in the text were also made by three-part ligations with the same DNA fragments used to construct pDPA/148tk except the *DPA* promoter fragments were replaced by double-stranded synthetic oligonucleotides. All annealed oligonucleotides had both *Xba* I and *Bam*HI sticky ends to facilitate constructions.

Transfection and hGH Assay. Eight hours after DNA transfection (21) with 2×10^6 HeLa cells and 15 μg of plasmid DNA, the cells were washed with DMEM once and IFN-γ was added to the dishes at 500 units/ml. The hGH assay (16) was performed 24–48 hr after IFN-γ treatment. Unlike the chloramphenicol acetyltransferase assay, the hGH assay

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Abbreviations: MHC, major histocompatibility complex; IFN, interferon; IRE, IFN-γ response element(s); hGH, human growth hormone; tk, thymidine kinase.

does not kill the cells. Therefore, total cellular RNA from untreated cells or from cells treated with IFN-γ was simultaneously isolated 48 hr after IFN-γ treatment.

Antisense RNA Probes and RNase Protection Analysis. The plasmids pSP37, pSP6-actin, and pSPtkIf have been described (22–24). The pSP3-GHL (long probe, not containing any promoter sequences) was constructed by insertion of a 550-bp *Hin*dIII/*Bgl* II fragment of hGH cDNA at the *Hin*dIII and *Bgl* II digested pSP73. The linear DNA template of pSP3-GHL for synthesis of antisense RNA probes was obtained by cleavage with *Hin*dIII. The plasmid pSP3-GHS (short probe including promoter sequences) was made by ligating a *Kpn* I/*Pst* I fragment from pDPA/148 into pSP73 vector. The linear template of pSP-GHS was made by *Kpn* I digestion. Uniformly labeled RNA probes were transcribed *in vitro* by SP6 or T7 polymerase from linearized DNA.

RNA·RNA hybridization was performed at 45°C for 12 hr in 400 mM NaCl/40 mM Pipes, pH 6.4/10 mM EDTA/80% formamide and digestion with RNase A (42 mg/ml) was carried out at 30°C for 1 hr in 300 mM NaCl/10 mM Tris·HCl, pH 7.5/5 mM EDTA. The digested RNA products were separated by electrophoresis through 6% denaturing gels (22).

RESULTS

The 5′ Boundary of the IFN-γ Response Region Defined by 5′ Deletions of the *DPA* Promoter. In preliminary experiments, the *DPA* promoter fragment from −148 to −11 bp subcloned into pDPA/O to give pDPA/148 gave near maximum activity (as compared to the entire 2.3-kilobase DNA sequence, which separates the 5′ ends of the *DPA* and *DPB* genes) of the reporter gene hGH (see *Materials and Methods* and, for details, ref. 25). A series of fine 5′ deletion mutants from −148 bp toward the transcriptional start site was generated by BAL-31 nuclease digestion of plasmid pDPA/148 (Fig. 1*A*) transfected into HeLa cells and IFN-γ induced. A critical position for the 5′ boundary of the IFN-γ response region was evident at position −107 of the *DPA* promoter. Further deletion to position −102 or −81 abolished IFN-γ inducibility (Fig. 1*B*). In contrast to other class II promoters, the 5′ boundary of the IFN-γ response region of the *DPA* promoter was very close to the X box. Interestingly, the difference between deletion constructs pDPA/107 and pDPA/102 was only 5 bp. However, pDPA/107 could be induced by IFN-γ, while pDPA/102 could not. The IFN-γ response region of the *DPA* promoter may overlap the X box, and/or the X box itself may be also indispensable for IFN-γ induction. To validate the results from the hGH assay, 15 μg of RNA isolated from the transfectants was hybridized with the hGH antisense RNA probes made from pSP3-GHS followed by digestion with RNase A. The same protected 220-nucleotide hGH RNA fragment was produced from the constructs pDPA/148, pDPA/117, and pDPA/107 in response to IFN-γ treatment (Fig. 1*C*; lanes 2, 4, and 6), indicating also that all initiated at the same point, but not from constructs pDPA/102 and pDPA/81 (lanes 8 and 10). RNase protection was consistent with hGH production. Thus, the 5′ boundary of the IFN-γ response region of the *DPA* promoter was located immediately upstream of the X box at position −107 of the *DPA* gene. In addition, a consistent 30% decrease of IFN-γ inducibility was observed by deletions at positions −123, −117, and −107 bp both by assay of hGH production (Fig. 1*B*) and by RNase protection analysis (Fig. 1*C*, compare lanes 4 and 6 with lane 2) (see below).

Identifying the 3′ Boundary of the IFN-γ Response Region in the *DPA* Promoter by 3′ Deletions. A set of 3′ deletion constructs was derived by BAL-31 nuclease digestion of the plasmid pDPA/148 starting from the *Bam*HI site at position −11 (Fig. 2*A*) and, after repair of the hGH gene, the constructs were transfected into HeLa cells and tested for IFN-γ inducibility. Deletions from positions −11 to −55 did not drastically change IFN-γ inducibility (although the 50% loss of activity at positions −28 and −55 may suggest that some sequence in the proximal region has some role). However, deletion at −82 bp or further completely shut down inducibility (Fig. 2*B*). The conserved Y box, containing a reverse CCAAT sequence, resided in the critical region at −59 to −68 bp. The hGH assay was also confirmed at the RNA level by RNase protection assay (data not shown). The 3′ boundary of the IFN-γ response region was thus mapped at about position −55 of the *DPA* gene. The Y box seemed to be an essential component of the IFN-γ response region.

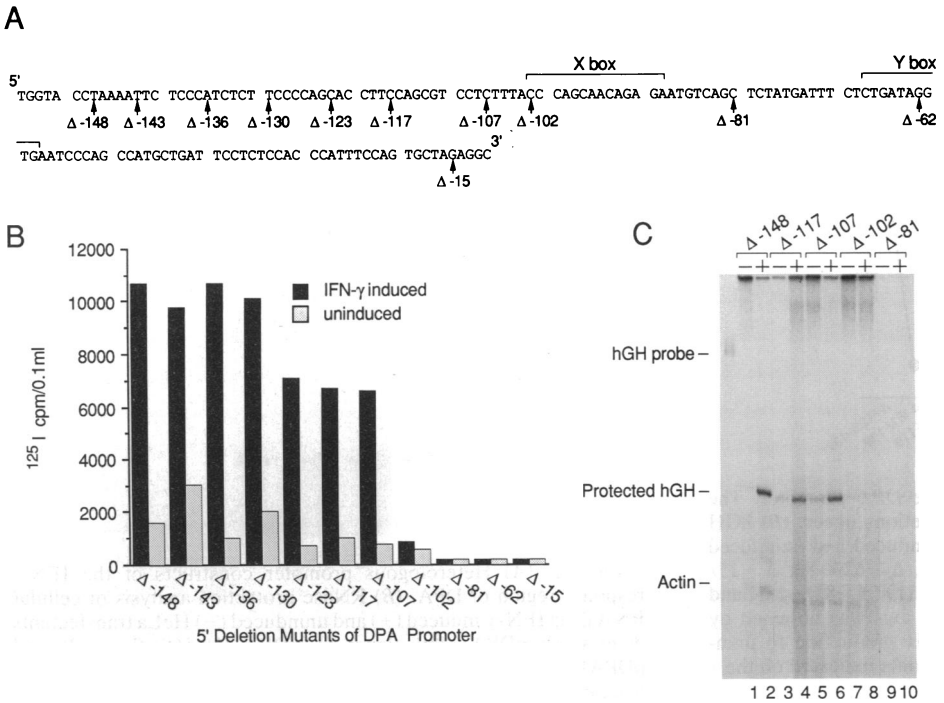

FIG. 1. (*A*) BAL-31 nuclease-generated 5′ deletion of the *DPA* gene from position −148. *Kpn* I-cut pDPA/148 was used as starting material for digestion with BAL-31. The class II gene conserved elements X and Y are shown in the immediate upstream sequence between positions −148 and −11 of the *DPA* gene. Deletion end points relative to the X and Y boxes are shown. (*B*) hGH assays of the 5′ deletion constructs in IFN-γ-induced and uninduced HeLa cells. (*C*) RNase protection analysis of total cellular RNA isolated from the IFN-γ-treated (+) and untreated (−) HeLa cells transfected with the plasmids pDPA/148, pDPA/117, pDPA/107, pDPA/102, and pDPA/81. The antisense RNA probes hGH and γ-actin (as internal control) were generated *in vitro* by SP6 polymerase from pSP-HGS and pSP6-actin (23). The protected hGH RNA fragment is ≈220 nucleotides.

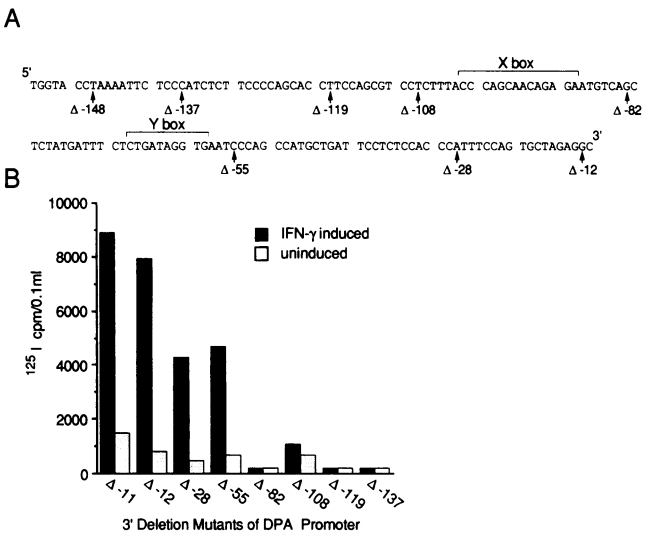

FIG. 2. (*A*) BAL-31 nuclease-generated 3′ deletion constructs made from *Bam*HI-digested pDPA/148. The deletion end points for these constructs are indicated by arrows. (*B*) hGH assays of 3′ deletion constructs in IFN-γ-induced and uninduced HeLa cells.

A Sequence of 5 Nucleotides Immediately Upstream of the Conserved X Box Is Important for IFN-γ Induction. Next, a set of gap deletion mutant constructs was made by simply joining the *Bgl* II/*Eco*RI DNA fragments from the 5′ deletion series and the *Bam*HI/*Eco*RI fragments from the 3′ deletion series (Fig. 3*A*). Gap deletions outside the IFN-γ response region between −55 and −107 bp did not affect IFN-γ inducibility—e.g., the gap deletions −15 to −55 bp or −123 to −137 bp. On the other hand, deletions inside −55 to −107 bp resulted in loss of IFN-γ inducibility (Fig. 3*B*). Most interestingly, the gap deletion, −102 to −108 bp, which had only a 5-bp deletion of nucleotides −103 to −107, abolished IFN-γ inducibility. This result is consistent with the 5′ deletion analysis where the construct pDPA/107 was induced while pDPA/102 was not. Thus, in addition to the X and Y boxes, a 5-mer sequence located immediately 5′ of the X box of the *DPA* gene within the critical −55- to −107-bp region was also required for an IFN-γ response.

A 52-bp DNA Fragment Containing Conserved X and Y Motifs and 5 Additional Nucleotides Are Required for IFN-γ Induction of a Heterologous Promoter. The characteristics of eukaryotic promoters may undergo subtle changes because of juxtaposition of plasmid vector sequences at the site of the deletion (26). To verify further the IFN-γ response region of the *DPA* gene and the roles of its individual elements (the X box, the Y box, and the 5-mer sequence), five double-stranded oligonucleotides with *Bam*HI and *Xba* I ends, the 10-mer DPA/108-99 (nucleotides −108 to −99), 24-mer DPA/108–85 (nucleotides −108 to −85), 32-mer DPA/110-79 (nucleotides −110 to −79), 23-mer DPA/78-56 (nucleotides −78 to −56), and 55-mer DPA/110-56 (nucleotides −110 to −56) were synthesized, fused to the tk promoter at the *Bam*HI site at position −115 (Fig. 4*A*), and transfected into HeLa cells. RNase protection assay (Fig. 4*B*) showed that IFN-γ-enhanced tk gene transcription was only observed in the constructs, pDPA/148tk, which contained a 137-bp *DPA* promoter fragment (−148 to −11) (lanes 1) and pDPA/110-56tk (lanes 2), both of which contained intact X and Y boxes as well as interspace sequences and upstream sequences. No IFN-γ induction was observed with other heterologous promoter constructs that contained only the X box sequence plus upstream sequences (pDPA/108-85tk) (lanes 3), or only the Y box sequence (pDPA/78-56tk), or the sequence between −107 and −102 bp (pDPA/108-99tk), or an even longer X box sequence including the 5′ 5-mer sequence (pDPA/110-79tk) (data not shown). Thus, the two conserved upstream sequences X and Y together with the 5 additional nucleotides

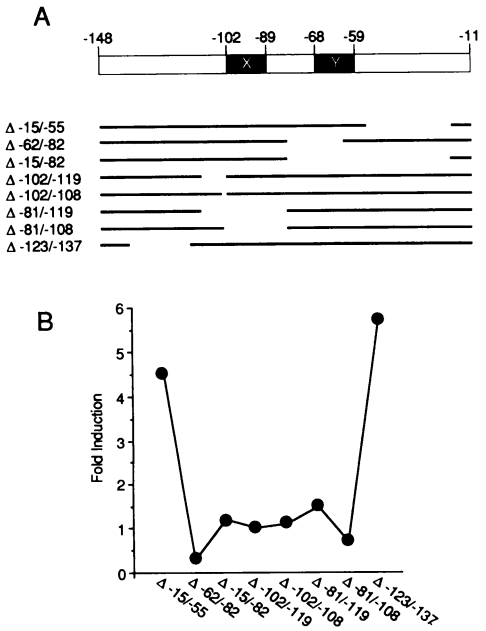

FIG. 3. (*A*) Gap deletion analysis of the *DPA* promoter. The schematic drawing illustrates where gap deletions occur. (*B*) hGH assays of gap deletion constructs in IFN-γ-induced and uninduced HeLa transfectants of plasmids Δ −15/−55, Δ −62/−82, Δ −15/−82, Δ −102/−119, Δ −102/−108, Δ −81/−119, Δ −81/−108, and Δ −123/−137. The -fold induction on the *y* axis was obtained by calculating the ratios of IFN-γ-induced hGH production to uninduced hGH production of the HeLa transfectants indicated on the *x* axis.

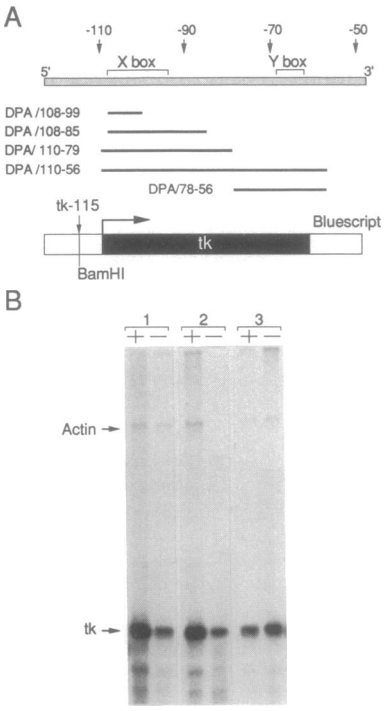

FIG. 4. (*A*) Heterologous promoter constructs of the IFN-γ response region of DPA. (*B*) RNase protection analysis of cellular RNA from IFN-γ-induced (+) and uninduced (−) HeLa transfectants of plasmids pDPA/148tk (lanes 1), pDPA/110-56tk (lanes 2), and pDPA/108-85tk (lanes 3). Antisense RNA probes were produced from pSPtkIf.

immediately 5′ to the X box were essential for IFN-γ induction of tk; none was dispensable.

DISCUSSION

The cis regulatory elements, which may control the inducibility of class II MHC genes by IFN-γ, have been investigated by several laboratories (12–15). The 5′ boundary of the IFN-γ response region has been defined in the *DQB* and *DRA* genes by 5′ deletion analysis, while the 5′ and 3′ boundaries have been mapped only in the *Eα* promoter (13). The last *inducible* 5′ deletion construct and the first *noninducible* 5′ deletion construct are the critical deletion sites (Fig. 5). In *DQB*, they were observed at positions −159 and −128 of the *DQB* 5′ flanking sequences (12), in *DRA* at −141 and −123 bp (14) or at −136 and −131 bp (15), in *Eα* at −164 and −150 bp (13) and in the present studies in *DPA* at −107 and −102 bp (Fig. 5). The 3′ end of the IFN-γ response region was mapped at −43 bp for *Eα* and at −55 bp for *DPA*.

The data obtained from these four class II promoters suggest that, in addition to the X and Y boxes, one or more short cis element(s) may be involved in IFN-γ-induced class II gene expression. DNA sequence comparison of these four promoters in the vicinity of their critical 5′ deletion sites revealed a conserved sequence, which we term γ-IRE. It has been found inside the IFN-γ response regions of all four promoters and is located in the vicinity of the critical deletion sites (Fig. 5). Specifically, it is located within the W box of the *DQB* 5′ flanking sequence, conserved among class II *B* genes (29); in the Z box of the *DRA* promoter (15); partially overlapped with the distal septamer sequence of the *DRA* gene (14); between position −142 and −135 of the *Eα* promoter (13); or between positions −107 and −102 of *DPA* overlapping the 5′ end of the X box (this paper). Importantly, when BAL-31 nuclease, used in 5′ deletion analysis, cut through or near this element, the IFN-γ inducibility of the promoter was abolished. Significantly, the 5-mer sequence found in the *DPA* gene together with 3 additional 5′ nucleotides of the X box was homologous to the regions previously described as IFN-γ response-related elements—i.e., Z box, septamer element, and W box in the *DRA* and *DQB* genes. The element may play an important role in the IFN-γ response region of all four promoters. However, although the 5′ end of the conserved X element includes position −102 and deletion after −101 might have damaged the X box, the juxtaposed *Bam*HI linker sequence (GGATCC) resulted in restoration of C at −102. Nevertheless, further studies by site-directed mutagenesis are needed to distinguish clearly the γ-IRE and the X box, particularly since at least one X box binding protein has been reported to footprint several nucleotides 5′ of the sequence defined as the X element (30). Recently, gel-shift assay using IFN-γ-induced or uninduced HeLa nuclear extracts has shown that a dimerized 7-mer sequence −109 to −103 from this region of the *DPA* gene had a characteristic nuclear protein binding pattern (L.W. and M.S., unpublished data).

Examination of our data and that for *DRA* (figure 4 in ref. 31 and figure 2 in ref. 32) and *Eα* (table 1 in ref. 15) suggests that sequential 5′ deletion results first in a partial loss of activity (as in *DPA*; Fig. 4) followed by abolition of activity with further deletion. Such data suggest that a second element important for IFN-γ induction may exist in the promoter regions of these genes. The sequences of this putative γ-IRE and a second element, which we term the W element, are compiled in Fig. 5. The γ-IRE may play a predominant role in responding to IFN-γ induction in *DPA*. Deletion or damage of the element resulted in a sharp reduction of IFN-γ inducibility of the gene, while deletion of the W element had only a partial effect. The W box in the *DQB* gene (29) contains both elements in the sequence γ-IRE–W juxtaposed. The *DRA* promoter has them in the same order but overlapping; an alternative W element is also present in *DRA* separated by 8 bp from the putative γ-IRE. In *Eα* they are separated by 27 nucleotides. In *DPA*, the order of the elements is reversed and they are separated by 12 nucleotides. The W element has previously been reported as a conserved element in the promoter regions of class II genes located 15–17 bp 5′ of the X box without evidence of function (27). Both elements overlap almost exactly a previously observed imperfect tandem repeat in the *DRA* promoter (33). The W element may also be required for IFN-γ inducibility (i.e., a second γ-IRE). More likely, however, it may be an element utilized in constitutive expression in B cells, which in *DPA*, at least, also augments the effect of the γ-IRE at nucleotides −107 to −102 (including the three 5′ nucleotides of the X box itself).

Cis-elements in other genes that are predominantly responsible for IFN-α, -β induction are also responsive to IFN-γ induction, e.g., the IRE of the 9-27 gene (28), the IRE of MHC class I genes (34, 35), and the PRDI of the IFN-β gene (36), although the two types of IFN recognize different receptors and may activate genes in a quite different way (7, 37, 38). A comparison of these sequences with the sequences reported for the 9-27 gene and other genes that are inducible by both IFN-α, -β, and -γ (28) is instructive (Fig. 5). Again, a

Gene	γ-IRE [II]	Location (bp)	Remarks	W element [I]	Location (bp)	Remarks
Ea	CTTGAAAT	-152 to -145	inside 5′ cds	AGGACCTG	-118 to -111	near 5′ cds
DQB	CTGGATTC	-146 to -139	in W box	AGAACCCT	-138 to -131	near 5′ cds
DRA	CTGGACCC	-133 to -126	in Z box	TGGACCCT	-132 to -125	near 5′ cds
				(AGAACCCT)	(-119 to -112)	
DPA	CTTTACCC	-107 to -100	inside 5′ cds	AGCACCTT	-125 to -118	near 5′ cds
γ-IRE consensus	$CT^{GG}_{TT}ANN^{C}_{T}$			$^{A}_{T}GNACC^{CG}_{TT}$		
9-27	CAGGAAAT	-173 to -167		AGAAACTT	-166 to -160	in IRE
α,β-IRE consensus	CAGGAAAN			NGAAACTT		
IRE consensus	$C^{AGG}_{TTT}ANN^{C}_{T}$			$^{A}_{T}GNA^{A}_{C}C^{CG}_{TT}$		

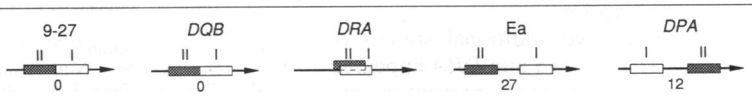

FIG. 5. Proposed conserved elements involved in IFN-γ induction. Conserved elements in the vicinity of critical 5′ deletion sites (cds) of the four MHC class II promoters were located by sequence comparisons. γ-IRE (II in the diagram) and the W element (I in the diagram), which could also play a role in IFN-γ induction, are indicated above the diagrams. The numbers below refer to the number of nucleotides between those two putative elements. The space between the 3′ end of the W element and the 5′ end of the X box in *DQB*, *DRA*, *Eα*, and *DPA* is 17, 15, 17, or 15 nucleotides, respectively. Two closely related W elements (one of which is shown in parentheses) are found in *DRA* (see refs. 14 and 27). γ-IRE is downstream of W only in the case of *DPA* and overlaps the X box by 3 nucleotides. α-, β-IRE consensus is from ref. 28.

conserved sequence of 23 nucleotides for those genes may be separable into two elements, each with some homology to γ-IRE and the W element in the promoter regions of the class II genes.

In addition, the conserved X and Y boxes are essential components of the IFN-γ response region of all four class II promoters . Since the Y boxes of all class II genes contain a reverse CCAAT motif, the role of the Y box may be the same as the CCAAT motifs in most eukaryotic promoters. However, CCAAT motifs from different promoters may bind different nuclear proteins, suggesting that the CCAAT motifs may have diverse functions (39). Sequences surrounding CCAAT motifs from different promoters may determine the specificity for DNA binding factors. Therefore, the Y boxes may have specific roles in class II gene regulation—i.e., they are not likely to be replaceable by CCAAT motifs from other promoters. Indeed in *Eα* transgenic mice, the Y box of the *Eα* gene was essential for induction of this gene by IFN-γ (40). In the present study, the Y box of the *DPA* gene was clearly indispensable for IFN-γ inducibility.

The X boxes of the class II genes play a crucial role in directing the cell-type-specific expression of the class II antigens (11, 15, 41, 42) and are also important components of the IFN-γ response (15). Thus, the elements X and Y although they appear to be sufficient as a B-cell-specific enhancer, at least with some promoters (11), may functionally cooperate with the γ-IRE and W to respond to IFN-γ treatment. Similarly, in the class I MHC gene promoter the element(s) involved in IFN-α, -β, and -γ induction is active only in the presence of another element, the class I MHC enhancer (34, 35). Our data also suggest that the cis elements γ-IRE and W are necessary but not sufficient for IFN-γ induction of class II MHC gene expression. None of the data excludes the possibility that γ-IRE and W may also play some role in constitutive expression in B cells.

In linker scanning mutations of *Eα* (43) deletion of the W element (termed the S element by these authors and the Z element in ref. 44) resulted in loss of IFN-γ inducibility in fibroblasts and loss of constitutive expression in B cells. These authors concluded that this element at positions −111 to −123 in *Eα* is required both for IFN-γ induction and for constitutive expression in B cells. Furthermore, a linker scanning deletion including positions −152 to −145 (γ-IRE of *Eα* in Fig. 5) had no effect on IFN-γ inducibility. These data are not entirely concordant with those presented in this paper and in a previous publication from this laboratory using other genes. Thus, the 5′ deletion and gap deletion mutants in *DPA* both show that the region of −125 to −118 (homologous to the W element) has at most a small role in IFN-γ inducibility of *DPA*. Furthermore, the X and Y element of the *DQB* genes showed full B-cell-specific enhancer activity on an enhancerless simian virus 40 promoter (11) in the absence of any components of the W box. These discrepancies need to be resolved by further experimentation. It should also be emphasized that the delineation of γ-IRE in this paper is based mainly on the 5′ and gap deletions of the *DPA* promoter and on sequence comparisons with other published data. Further analysis by such techniques as DNA footprinting, methylation interference analysis, and site-specific mutagenesis will serve to validate or invalidate these elements and to define more accurately their precise locations.

After this paper was written, two additional studies of IFN-γ induction of the class II genes *Aα* and *DRA* appeared (44, 45). The data are compatible with those presented here and will be discussed more fully in a later publication.

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1. Benacerraf, B. (1981) *Science* **212**, 1229–1238.
2. Kaufman, J. F., Auffray, C., Korman, A. J., Shackelford, D. A. & Strominger, J. L. (1984) *Cell* **36**, 1–13.
3. Collins, T., Pober, J. S. & Strominger, J. L. (1986) in *Human Class II Histocompatibility Antigens: Theoretical and Practical Aspects–Clinical Relevance*, eds. Ferrone, S., Solheim, B. G. & Moller, E. (Springer, Berlin), pp. 14–31.
4. Paul, W. E. & Ohara, J. (1987) *Annu. Rev. Immunol.* **5**, 429–459.
5. Kappes, D. & Strominger, J. L. (1988) *Annu. Rev. Biochem.* **57**, 991–1028.
6. Collins, T., Korman, A. J., Wake, C. T., Boss, J. M., Kappes, D. J., Fiers, W., Ault, K. A., Gimbrone, A. M., Jr., & Strominger, J. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4917–4921.
7. Blanar, M. A., Boettger, E. C. & Flavell, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4672–4676.
8. Amaldi, W., Reith, W., Berte, C. & Mach, B. (1989) *J. Immunol.* **142**, 999–1004.
9. Okada, K., Prentice, H. L., Boss, J. M., Levy, D. J., Kappes, D., Spies, T., Raghupathy, R., Mengler, R. A., Auffray, C. & Strominger, J. L. (1985) *EMBO J.* **4**, 739–748.
10. Kelley, A. & Trowsdale, J. (1985) *Nucleic Acids Res.* **13**, 1607–1621.
11. Sakurai, M. & Strominger, J. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6909–6913.
12. Boss, J. M. & Strominger, J. L. (1986) *Proc. Natl. Acad. Sci. USA* **82**, 9139–9143.
13. Thanos, D., Mavrothalassitis, G. & Papamatheakis, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3075–3079.
14. Basta, P. V., Sherman, P. A. & Ting, J. P.-Y. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8618–8622.
15. Tsang, Y. S., Nakanishi, N. & Peterlin, B. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8598–8602.
16. Selten, R. F., Howie, K. B., Rowe, M. E., Goodman, H. M. & Moore, D. D. (1986) *Mol. Cell. Biol.* **6**, 3173–3179.
17. Okada, K., Boss, J., Prentice, H., Spies, T., Mengler, R. & Strominger, J. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3410–3414.
18. Kappes, D. (1988) Ph.D. thesis (Harvard Univ., Cambridge, MA).
19. McKnight, S. L. & Kingsbury, R. (1982) *Science* **217**, 316–324.
20. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Chen, C. & Okayama, H. (1987) *Mol. Cell. Biol.* **7**, 2745–2752.
22. Krieg, P. & Melton, D. (1987) *Methods Enzymol.* **155**, 397–415.
23. Enoch, T., Zinn, K. & Maniatis, T. (1986) *Mol. Cell. Biol.* **6**, 801–810.
24. Goodbourne, S., Zinn, K. & Maniatis, T. (1985) *Cell* **41**, 509–520.
25. Yang, Z. (1989) Ph.D. thesis (Harvard Univ., Cambridge, MA).
26. Widera, G., Burkly, L. C., Pinkert, C. A., Bottger, E. C., Cowing, C., Palmiten, R. D., Brinster, R. L. & Flavell, R. A. (1987) *Cell* **51**, 175–187.
27. Servenius, B., Rask, L. & Peterson, P. (1987) *J. Biol. Chem.* **262**, 8759–8766.
28. Reid, L. E., Brasnett, A. H., Gilbert, C. S., Porter, A. C. G., Gewert, D. R., Stark, G. R. & Kerr, I. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 840–844.
29. Miwa, K., Doyle, C. & Strominger, J. L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4939–4943.
30. Reith, W., Barras, E., Satola, S., Kobr, M., Reinhart, D., Herrero Sanchez, C. H. & Mach, B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4200–4204.
31. Blanck, G. & Strominger, J. L. (1990) *Hum. Immunol.* **27**, 265–268.
32. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
33. Schamboeck, A., Korman, A. J., Kamb, A. & Strominger, J. L. (1983) *Nucleic Acids Res.* **11**, 8663–8674.
34. Israel, A., Kimura, A., Fournier, A., Fellous, M. & Kourilsky, P. (1986) *Nature (London)* **322**, 743–746.
35. Blanar, M. A., Baldwin, A. S., Jr., Flavell, R. A. & Sharp, P. A. (1989) *EMBO J.* **8**, 1139–1144.
36. Fang, C. M. & Maniatis, T. (1989) *EMBO J.* **8**, 101–110.
37. Dianzani, F., Zucca, M., Scupham, A. & Georgiades, J. A. (1980) *Nature (London)* **283**, 400–402.
38. Rutherford, M. N., Hanniagan, G. E. & Williams, B. R. G. (1988) *EMBO J.* **7**, 751–759.
39. Dorn, A., Bollekens, J., Staub, A., Benoist, C. & Mathis, D. (1987) *Cell* **50**, 863–872.
40. Dorn, A., Durand, B., Marfing, C., Le Meur, M., Benoist, C. & Mathis, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6249–6253.
41. Sloan, J. & Boss, J. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8186–8190.
42. Sherman, P., Basta, P. & Ting, J. P.-Y. (1989) *Mol. Cell. Biol.* **9**, 50–56.
43. Benoist, C. & Mathis, D. (1990) *Annu. Rev. Immunol.* **8**, 681–715.
44. Tsang, S. Y., Nakanishi, M. & Peterlin, B. M. (1990) *Mol. Cell. Biol.* **10**, 711–719.
45. Dedrick, R. L. & Jones, P. P. (1990) *Mol. Cell. Biol.* **10**, 593–604.