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B-cell-specific and interferon-γ-inducible regulation of the *HLA-DRα* gene

(DRα promoter/class II major histocompatibility complex genes/gene regulation)

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ABSTRACT **We investigated the cis-acting sequences that function in the B-cell-specific and interferon-γ-inducible expression of the *HLA-DRα* gene, a human class II major histocompatibility complex gene. The effects of 5′ deletions on the activity of the DRα promoter and the influence of upstream DRα promoter elements on the activity of the herpes simplex virus thymidine kinase promoter were examined by a transient transfection assay in human B-, T-, and fibroblast cell lines. We show that the *DRα* gene is regulated by positive and negative cis-acting sequences between positions −1300 and +31 from the site of initiation of transcription. We also demonstrate that the DRα promoter sequences from positions −116 to −92 and from −136 to −80 are the minimal sequences required for conferring B-cell specificity and interferon-γ inducibility upon the Herpes simplex virus thymidine kinase promoter, respectively.**

The class II genes (*DP*, *DQ*, and *DR*) of the human major histocompatibility complex encode proteins that are expressed on the surfaces of immunocompetent cells. These class II molecules play an essential role in the regulation of the immune response (see refs. 1–3 for review). Three different patterns of class II gene expression are found. High levels of expression are seen in mature B cells, activated or virally infected T cells, and thymic epithelial cells. Class II genes are inducible by interferon-γ (IFN-γ) in antigen-presenting cells, melanoma cells, and some fibroblasts, and by interleukin 4 in pre-B cells. However, in most somatic cells, class II genes are not expressed nor can their expression be induced by these lymphokines (3).

Several cis-acting elements that regulate class II gene expression have been described. Lymphoid cell-specific transcriptional enhancers were found in the 5′ flanking region of the Eβ gene (4), in the body and 5′ flanking regions of the *DRα* and *DQα* genes (5, 6), and in the 3′ coding region of the *DQβ* gene (6). In addition, upstream promoter sequences were shown to be involved in both IFN-γ-inducible and B-cell-specific expression of class II genes. Sequences from positions −159 in the DQβ promoter (7) and sequences from positions −267 to +31 in the DRα promoter (8) were found to be sufficient for IFN-γ inducibility. Furthermore, a single DNase I hypersensitive site, which may correspond to a site of interaction with DNA-binding regulatory proteins, was mapped into the upstream promoter region of the *DRα* gene in both resting and IFN-γ-induced macrophages and HeLa cells (9). These studies strongly suggest that the cis-acting elements involved in the response to IFN-γ lie in the upstream promoter region of the class II genes. Sequences from positions −109 to +31 in the DRα were reported to be sufficient for B-cell-specific expression (10). However, DNase I hypersensitivity studies in B cells revealed two additional DNase I hypersensitive sites (9). They were mapped into the first intron of the *DRα* gene where a lymphoid cell-specific transcriptional enhancer is located. Therefore, B-cell-specific expression of the *DRα* gene is likely to result from cooperative interactions between the promoter and the intronic transcriptional enhancer.

Three conserved upstream sequences (CUS), which are observed in all class II genes and may play an essential role in the transcriptional regulation of these genes, have been described (11, 12). In the DQβ promoter, they are called the W-, X-, and Y-boxes and are located between positions −142 to −127, −113 to −100, and −80 to −67, respectively (13). In the DRα promoter, they are located from positions −135 to −61. We call them the Z-, X-, and Y-boxes. In this study, the function of the CUS in the B-cell-specific and IFN-γ-inducible expression of the DRα promoter was analyzed. We show here that sequences from positions −136 to +31 are sufficient for the appropriate expression of the DRα promoter in human B, T, and IFN-γ-inducible cells. Furthermore, sequences from positions −116 to −92 and from −136 to −80 confer B-cell specificity and IFN-γ inducibility upon the Herpes simplex virus thymidine kinase (TK) promoter (14), respectively.

MATERIALS AND METHODS

Cell Culture. Raji (ATCC CCL86) is a human Epstein–Barr virus-positive Burkitt lymphoma B-cell line that expresses high levels of DR determinants. Jurkat is a human Sezary T-cell line that does not express class II antigens. Their expression cannot be induced in our clone of Jurkat either by the administration of T-cell agonists or by transfection with viral trans-activators. HeLa (ATCC CCL2) is a cervical carcinoma cell line. While resting HeLa does not express DR determinants, their expression can be induced by the administration of IFN-γ. Raji and Jurkat were grown in RPMI 1640 medium supplemented with penicillin, streptomycin, and fetal calf serum (20% for Raji, 10% for Jurkat cells). Adherent HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

Plasmid Constructions. The control plasmid pRSVCAT [Rous sarcoma virus long-terminal repeat linked to the chloramphenicol acetyltransferase (CAT) gene (15)] has been described (16). The ptkCAT plasmid (pTE2) was constructed by inserting into PUC18 a 2.3-kilobase *Hin*dIII/*Bam*HI fragment containing a polylinker sequence, the TK promoter (positions −109 to +51), and the CAT gene from pTE1 (17).

The parental plasmid (pTE2Δtk) used for the cloning of all 5′ DRα promoter deletions was derived from pTE2 by deleting the −160-base-pair *Sac* I/*Sac* I fragment, which contains the TK promoter. Deletion constructions p(5′ −1300), p(5′ −268), p(5′ −80), and p(5′ −67) were prepared

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Abbreviations: IFN, interferon; CUS, conserved upstream sequence(s); CAT, chloramphenicol acetyltransferase; TK, thymidine kinase.

by inserting the appropriate restriction fragments of the *DRα* gene into polylinker cloning sites of pTE2Δtk upstream of the CAT gene (see Fig. 1). Additional deletion mutants p(5′ −150), p(5′ −136), p(5′ −131), and p(5′ −106) were obtained by BAL-31 digestion of the plasmid p(5′ −268). Plasmid p(5′ −96) was obtained by BAL-31 digestion of plasmid p(5′ −106).

Plasmid ptkCAT (pTE2) was the parental plasmid used in all DRα-tkCAT constructions (see Fig. 3). DRα promoter sequences −136 to −80 and −136 to −29 were isolated from p(5′ −136). Oligonucleotides corresponding to the DRα sequences from positions −136 to −107, −136 to −116, −116 to −82, −116 to −92, −116 to −104, −110 to −61, −109 to −92, and −85 to −60 were synthesized. Most oligonucleotides were synthesized with an *Xba* I site at their 5′ and a *Bam*HI site at their 3′ ends. These oligonucleotides were cloned into the pTE2 vector upstream of the TK promoter. All manipulations were done by standard techniques (18). The deletions and oligonucleotide constructions were verified by DNA sequencing.

Transient Transfections and CAT Assays. All transfections were performed as described by the DEAE-dextran/chloroquine bisphosphate technique (19). After transfection, cells were incubated for 48 hr in culture medium with or without recombinant IFN-γ (50 units/ml) (Genentech, South San Francisco). Cell lysates were then prepared and assayed for CAT activities as described (15, 20). At least three separate transfections were done with each plasmid construction. In addition to the test plasmids, each set of transfections included pRSVCAT and ptkCAT as positive and negative controls, respectively. Transfection efficiencies were evaluated by comparing the CAT activities obtained with ptkCAT and pRSVCAT in different cells.

RESULTS

Deletion Analysis of the DRα Promoter: B-Cell Specificity and IFN-γ Inducibility. To map important regulatory sequences in the DRα promoter, we constructed plasmids that contain progressive 5′ deletions from positions −1300 to −67 relative to the DRα site of initiation of transcription, linked to the CAT reporter gene (Fig. 1). These plasmids were transfected into Raji, Jurkat, and HeLa cells. CAT enzymatic activities were assayed.

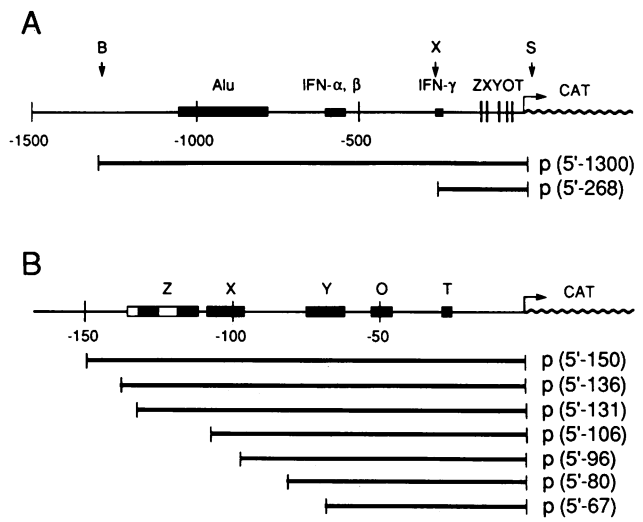


FIG. 1. Map of 5′ deletions. (*A*) The *DRα* gene from position −1500 to +31 (*Sac* I site). The previously described *Alu* repeat positions (−1040 to −780) (21), the IRS (IFN-α, β, positions −593 to −565) (22), and the IFN-γ consensus sequence (IFN-γ, positions −264 to −256) (8) are diagrammed. The deletions are to *Bgl* II (position −1300) and *Xba* I (position −268) sites, respectively. B, X, and S above arrows, *Bgl* II, *Xba* I, and *Sac* I sites, respectively. Z, X, Y, O, and T, the Z-, X-, Y-boxes, octamer, and TATA box. Horizontal arrow, site of initiation of transcription. (*B*) Deletions past the *Xba* I site.

In Raji cells, which express high levels of class II determinants, 5′ deletions from positions −1300 to −136 gave CAT enzymatic activities 2- to 3-fold above those obtained with the positive control plasmid pRSVCAT (Fig. 2*A*). In contrast, in Jurkat and resting HeLa cells, which do not express class II determinants, these deletions gave CAT activities that were even lower than those observed with the negative control plasmid ptkCAT (Fig. 2 *B* and *C*). The administration of IFN-γ did not affect the CAT activities of these plasmids in Raji and Jurkat cells (data not shown), whereas in HeLa cells, IFN-γ administration resulted in 4- to 7-fold increased CAT activities (Fig. 2*C*). IFN-γ administration did not affect the expression of the control plasmids pRSVCAT and ptkCAT (Fig. 2).

Deletion of an additional 5 nucleotides to position −131 resulted in a CAT activity decreased by a factor of 7 in Raji cells, and in a 20-fold and 3-fold increased CAT activity in Jurkat and HeLa cells, respectively (Fig. 2). When differences in transfection efficiencies are taken into consideration, the CAT activity of this plasmid in Raji and Jurkat cells was comparable. In HeLa cells, this plasmid was not inducible by IFN-γ. Further deletions beyond position −131 resulted in low CAT activities in Raji, Jurkat, and HeLa cells. In HeLa cells, these deletions were also not inducible by

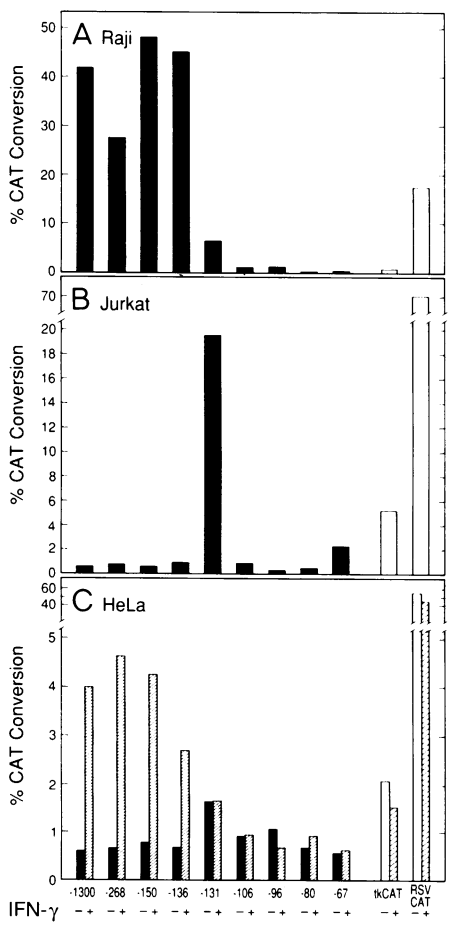


FIG. 2. CAT enzymatic activities of the DRα deletions. Data are expressed as % chloramphenicol conversion to its monoacetylated forms. Each bar represents the arithmetic mean of at least three independent transfections. SEM was <30% (data not shown). (*A*) Raji cells. (*B*) Jurkat cells. (*C*) HeLa cells. Solid bars, resting HeLa cells; hatched bars, HeLa cells induced by IFN-γ. − and +, absence and presence of IFN-γ, respectively.

IFN-γ (Fig. 2). These results show that the sequences responsible for B-cell specificity and IFN-γ inducibility reside downstream of position −136. Furthermore, they suggest that sequences from positions −136 to −131 interact with a positive factor in B cells and with a negative factor in Jurkat and HeLa cells. In HeLa cells, this negative regulation is apparently removed via intracellular signals induced by IFN-γ.

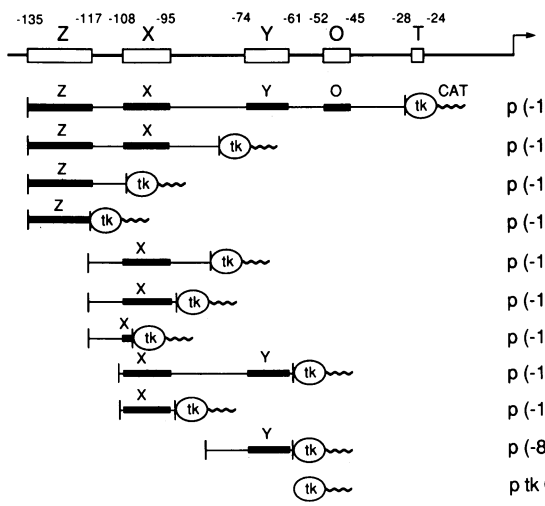
DNA Fragments from the DRα Promoter Confer B-Cell Specificity and IFN-γ Inducibility upon the TK Promoter. To further map B-cell-specific and IFN-γ-inducible sequences in the DRα promoter, oligonucleotides containing portions of the DRα promoter from positions −136 to −29 were placed upstream of the TK promoter and CAT gene (Fig. 3). These plasmids were tested in the same transient expression assay. The results are expressed as CAT activity relative to the value obtained with the TK promoter alone (Fig. 3). In Raji cells, only 4 of the 10 DRα-tk CAT constructions tested resulted in high relative CAT activities. These are p(−136/−29)tk, which contains Z-, X-, Y-boxes and the octamer ATTTGCAT (23, 24); p(−136/−80)tk, which contains Z- and X-boxes; and p(−116/−82)tk and p(−116/−92)tk, which both contain the X-box and its flanking sequences. The CAT activities of p(−136/−29)tk, p(−116/−82)tk, and p(−116/−92)tk were comparable and were 64-, 58-, and 46-fold over the control TK promoter, respectively. The relative CAT activity for p(−136/−80)tk was, on the average, 2- to 3-fold higher than that for either p(−136/−29)tk, p(−116/−82)tk, or p(−116/−92)tk.

Unlike the four plasmids described above, constructions that contain the Z-box [p(−136/−107)tk and p(−136/−116)tk], the 5′ flanking sequences of the X-box [p(−116/−104)tk], the X-box with no 5′ flanking sequences [p(−109/−92)tk], or the Y-box [p(−85/−60)tk], all gave low relative CAT activities ranging from ≈1- to 4-fold over the TK promoter in Raji cells. Comparing to constructions that contain either the X-box or the Y-box alone [p(−109/−92)tk or p(−85/−60)tk], a slightly higher CAT activity was observed for p(−110/−61)tk, which contains both the X- and Y-boxes.

In contrast to the results obtained in Raji cells, all oligonucleotide constructions, including those containing the Z- and X-boxes and those containing the X-box and its 5′ flanking sequences, gave low relative CAT activities in Jurkat and resting HeLa cells. In HeLa cells, IFN-γ inducibility was observed only with plasmids that contain both the Z- and X-boxes p(−136/−80)tk and p(−136/−29)tk. We conclude that sequences from positions −116 to −92 and −136 to −80 are both necessary and sufficient for conferring B-cell specificity and IFN-γ inducibility, respectively. Furthermore, these results strongly suggest the existence of positive and cooperative interactions between different DRα upstream promoter elements in B cells.

Analysis of the DRα Promoter Sequence. The DRα promoter contains several conserved sequences, some unique to class II genes and others shared with other gene families (Fig. 4*A*) (21, 25). Based on our results, sequences that are essential for B-cell specificity and IFN-γ inducibility reside from positions −116 to −92 and from positions −136 to −80, respectively (Fig. 5). The B-cell-specific region (from −116 to −92) contains one class II CUS, the X-box (located from positions −108 to −95), and a short stretch of highly pyrimidine-rich 5′ flanking sequence (11, 12). This pyrimidine-rich stretch is present in all class II genes studied. The IFN-γ-inducible region (from positions −136 to −80) contains two class II CUS, the Z- (located from positions −135 to −117) (12) and X-boxes. The sequences from positions −136 to −106, which include the Z-box, are 70% identical to an IFN-responsive sequence, defined by Friedman and Stark (22) (Fig. 4*B*), which is believed to be required for IFN-α and IFN-β inducibility. Sequences from positions −136 to −117 in the DRα promoter and the negative regulatory sequences described in the DQβ and IFN-β promoters contain a region of dyad symmetry (Fig. 4*C*) (7, 26). In the DQβ promoter, a perfect stem–loop structure can be formed from positions −159 to −141. Although the IFN-β gene responds to viral infections and poly(I)-poly(C), while class II genes respond to IFN-γ, these promoters could share common regulatory mechanisms.

Sequences downstream of position −80 show neither B-cell specificity nor IFN-γ inducibility. However, this



Plasmid Constructions	Relative CAT Activity				
	Raji	Jurkat	HeLa (−)	HeLa(+)	Fold Induction
p (-136/-29) tk	63.7±5.2	3.7±0.3	3.6±0.7	13.3±1.0	3.7
p (-136/-80) tk	119.0±31.0	1.4±0.4	5.7±1.0	20.0±3.0	3.5
p (-136/-107) tk	3.9±0.2	1.6±0.2	7.5±2.8	6.4±2.2	0.8
p (-136/-116) tk	1.3±0.4	1.3±0.5	2.6±0.1	2.7±0.2	1.1
p (-116/-82) tk	58.0±3.3	4.2±0.3	7.3±3.2	12.1±6.8	1.7
p (-116/-92) tk	45.5±0.8	2.0±0.2	6.4±1.3	7.7±2.2	1.2
p (-116/-104) tk	2.4±0.2	1.5±0.1	11.2±1.7	9.8±1.9	0.9
p (-110/-61) tk	7.2±0.2	1.4±0.1	7.5±3.1	7.6±2.0	1.0
p (-109/-92) tk	2.2±0.4	1.0±0.1	4.9±3.3	3.9±2.7	0.8
p (-85/-60) tk	1.0±0.2	1.1±0.2	4.1±0.2	4.1±0.7	1.0
p tk CAT	1.0±0.1	1.0±0.1	1.0±0.4	0.9±0.3	0.9

FIG. 3. Relative CAT activity of plasmids containing DRα synthetic oligonucleotides linked to the TK promoter in Raji, Jurkat, and HeLa cells. Maps of oligonucleotide constructions are shown on the left. The DRα upstream promoter sequences are diagrammed above the plasmid constructions. Open circle, TK promoter; wavy line, CAT gene. Plasmids are named for their 5′ and 3′ ends. Relative CAT activity represents the % chloramphenicol conversion of plasmids containing the DRα oligonucleotide over the ptkCAT values. Each value represents the arithmetic mean ± SEM of at least three independent transfections. Values reported here for each cell line are derived from transfections done on the same day. Day to day variability between transfections was <30%. HeLa(−), resting HeLa cells; HeLa(+), HeLa cells induced by IFN-γ. Fold induction, value of the relative CAT activity in HeLa (+) cells over that of HeLa (−) cells; it is a measure of plasmid induction by IFN-γ.

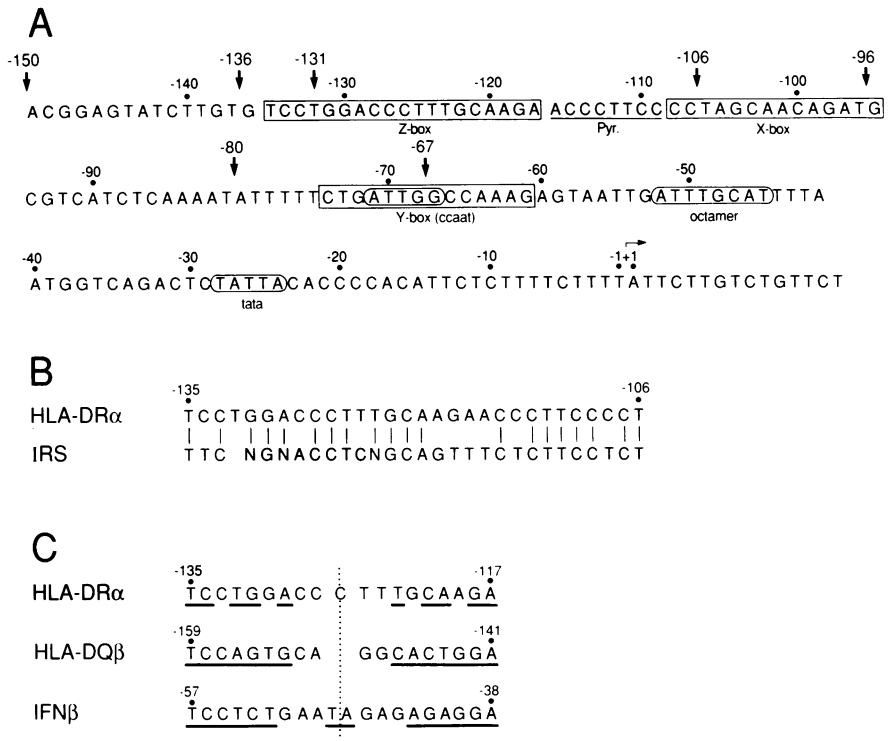


FIG. 4. Sequences of the DRα promoter. (*A*) Sequences from positions −150 to +15. Numbered arrows indicate the positions of the 5′ deletions used in the study. The class II CUS are boxed. The inverted CCAAT box, octamer, and TATA box are circled. The pyrimidine-rich stretch upstream of the X-box is underlined. (*B*) Comparison between DRα sequence from positions −135 to −106 and the IFN-responsive sequence (IRS) (22). (*C*) Comparison between DRα, DQβ, and IFN-β negative regulatory sequences. Regions of dyad symmetry are underlined.

region does contain several conserved sequences. The Y-box, which contains an inverted copy of the general transcriptional activator sequence CCAAT (27), is located between positions −74 and −61. The octamer (ATTTGCAT) and the TATA box (28) are located from positions −52 to −45 and −28 to −24, respectively (Fig. 4*A*). The octamer sequence has been shown to function as a lymphoid cell-specific element in the immunoglobulin genes (29, 30). However, in the DRα promoter, the deletion to position −67, which contains only the octamer and TATA sequences, gave low CAT activity in Raji, Jurkat, and HeLa cells. Thus, the DRα promoter can be divided into two functional units (Fig. 5). The sequences from positions −136 to −80 confer B-cell specificity and IFN-γ inducibility, while the sequences from position −80 to the cap site may determine accurate and efficient initiation of transcription from the DRα promoter.

DISCUSSION

We mapped positive and negative regulatory sequences in the DRα promoter. Using 5′ deletions, we demonstrated that DNA sequences from positions −136 to +31 are sufficient for both B-cell-specific and IFN-γ-inducible expression of the

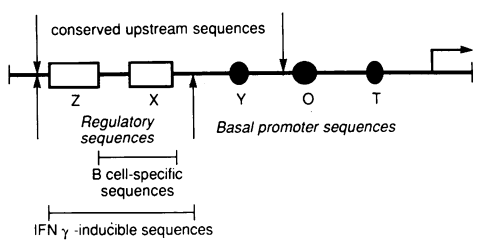


FIG. 5. Schematic representation of the DRα upstream promoter sequences. The Z- and X-boxes represent regulatory sequences and are drawn as open squares. The minimal sequences required for conferring B-cell specificity and IFN-γ inducibility are outlined below these boxes. The Y-box, octamer, and TATA box represent basal promoter sequences and those determining the site of initiation of transcription. They are drawn as solid circles. Horizontal arrow, site of initiation of transcription.

DRα gene. Experiments with synthetic oligonucleotides placed upstream of the TK promoter allowed us to further localize the B-cell-specific element to CUS from positions −116 to −92 and the IFN-γ-inducible element to CUS from positions −136 to −80. These CUS are found within a DNase I hypersensitive site, which suggests that they are open and accessible to regulatory DNA binding proteins (9, 31). The results reported here demonstrate the existence of several complex and cooperative interactions between the CUS. While both positive and negative regulation was observed in the context of the DRα promoter, the oligonucleotides containing portions of the DRα promoter conferred only positive regulation upon the TK promoter. We believe this might be due to protein–protein interactions in the context of the intact DRα promoter. Since the TK promoter binds different trans-acting factors and the distances between the CUS and TK cis-acting elements do not reflect the organization of the DRα promoter, these negative interactions were not observed with the hybrid promoters.

Studies of 5′ deletions and oligonucleotides reveal the existence of several important cis-acting regulatory elements in the DRα promoter. In Raji cells, the deletion of sequences from positions −136 to −131 reduced the high level of expression (Fig. 2). These 5 nucleotides may be a contact point for a positive trans-acting factor in B cells. Further deletion to position −106, which cuts into the X-box, decreased the activity to a barely detectable level. This suggests the presence of a second positive regulatory element located either between positions −131 and the X-box or in the X-box in B cells. Results of studies using oligonucleotides support the existence of two positive B-cell-specific elements between positions −136 and −80, one located in the Z-box from positions −136 to −116 and the other in the pyrimidine tract and the X-box from positions −116 to −92 (Fig. 3). However, the Z-box does not function by itself but requires the presence of the X-box for full activity (see below). Noting that the differences in the activities between p(−136/−80)tk and p(−116/−82)tk or p(−116/−92)tk were only 2- to 3-fold, whereas they were 7-fold between 5′ deletions to positions −136 and −131, we cannot exclude the possibility that either an additional negative regulatory element exists between

positions −131 and −116 or that the TK promoter fortuitously mimics part of the Z-box function.

In contrast to Raji cells, deletion of sequences from positions −136 to −131 resulted in increased levels of expression in Jurkat and resting HeLa cells (Fig. 2 *B* and *C*). The activity of deletion to position −131 was higher in Jurkat than in HeLa cells, which might be due to additional negative regulation 3′ to the X-box in HeLa cells (unpublished data). This increase in activity is unlikely to represent an artifact of the junctional sequence between the plasmid vector and inserted DRα DNA for several reasons. First, the deletion to position −131 resulted in decreased activity in Raji cells, where it was comparable to that observed in Jurkat cells. Second, similar increases in expression were described with a deletion from positions −159 to −128 in the DQβ promoter in human fibroblast cells (7). Finally, the examination of the junctional region revealed no sequence similarity with any known transcriptional enhancer element. Thus, nucleotides 3′ to position −136 are a likely contact point for a negative trans-acting factor in Jurkat and resting HeLa cells. Interestingly, this sequence is similar to the negative regulatory sequences in the DQβ and IFN-β promoters (Fig. 4) (7, 26). In HeLa cells, IFN-γ inducibility was lost with the deletion to position −131 and with all subsequent 5′ deletions. This suggests that some or all of the negative regulation in this region is relieved after administration of IFN-γ.

As revealed by oligonucleotide studies, complex and cooperative interactions exist between the various cis-acting regulatory elements in the DRα promoter. While both p(−116/−104)tk and p(−109/−92)tk resulted in low activities in all three cell lines, p(−116/−92)tk and p(−116/−82)tk yielded at least 20-fold higher activities in Raji cells (Fig. 3). Therefore, the cooperativity between the X-box and its immediate 5′ flanking sequence is required for the B-cell-specific expression of the *DRα* gene. p(−136/−80)tk resulted in an even higher level of expression, suggesting that the extended X-box and the Z-box interact in a B-cell-specific fashion. This is of special interest since p(−136/−116)tk and p(−136/−107)tk, which contain the Z-box, resulted in low activities in all three cell lines. The activity of p(−110/−61)tk was also more than the sum of those for p(−109/−92)tk and p(−85/−60)tk in Raji cells, implying cooperativity between the X- and Y-boxes. In contrast, IFN-γ inducibility was seen only with p(−136/−80)tk and p(−136/−29)tk (Fig. 3). Both oligonucleotides contain the Z- and X-boxes. IFN-γ inducibility of the *DRα* gene requires the cooperative interactions between these CUS, and neither the Z-box nor the extended X-box alone is sufficient for this response.

We do not know whether all the 5′ deletions were initiated correctly since their level of expression was insufficient for mRNA analysis in our transient expression assay. However, in studies with the DQβ promoter, 5′ deletions to and slightly past the Y-box were initiated correctly (7). In our own study, the oligonucleotide constructions used the TK cap site (ref. 5; data not shown).

We mapped the B-cell-specific and IFN-γ-inducible elements into 25 and 56 overlapping nucleotides of the DRα promoter, respectively. Our data suggest that these regions are further subdivided into positive and negative regulatory sequences, which function differently in the three phenotypically distinct modes of *DRα* gene expression. Further insights into the regulation of class II genes in general and of the DRα promoter in particular will come from studies in which clustered mutations are systematically introduced into sequences from positions −136 to +31 and in which protein interactions with these upstream promoter sequences are revealed.

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