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Arnulf Dorn, Beatrice Durand, Corinne Marfing, Marianne Le Meur, Christophe Benoist, Diane Mathis

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Immunology

Conserved major histocompatibility complex class II boxes—X and Y—are transcriptional control elements and specifically bind nuclear proteins

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ARNULF DORN, BÉATRICE DURAND, CORINNE MARFING, MARIANNE LE MEUR, CHRISTOPHE BENOIST, AND DIANE MATHIS

Laboratoire de Génétique Moléculaire des Eucaryotes, Centre National de la Recherche Scientifique, Unité 184 de Biologie Moléculaire et de Génie Génétique, Institut National de la Santé et de la Recherche Médicale, Institut de Chimie Biologique, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg Cedex, France

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ABSTRACT **A conserved sequence motif exists at the 5′ end of all major histocompatibility complex class II genes. This motif consists of the 14-base X and Y boxes separated by a short stretch of variable sequence. In this report, we provide evidence that the X and Y boxes play an important role in controlling transcription of the murine class II gene E^k_α. We have developed transgenic mouse lines that carry E_α genes cleanly deleted for either the X or Y box and have compared the expression of these mutant transgenes with that of a nondeleted control. Both the X and the Y segments appear critical for accurate and efficient transcription of E^k_α. The most drastic effect is seen with γ-interferon-treated macrophages, where deletion of the Y box completely abrogates transcription initiated by the normal promoter. In addition, we identify proteins from nuclear extracts that bind specifically to the X or Y box.**

The murine Ia antigens are encoded by a family of genes—A_α, A_β, E_α, and E_β—that resides in the major histocompatibility complex (MHC) (1). These genes, known as class II loci, are expressed coordinately and are subject to a medley of regulatory influences (2). Accordingly, mouse class II molecules are limited primarily to immunocompetent cells—including B cells, antigen-presenting cells, and thymic epithelial and dendritic cells. Within a given lineage, expression varies with the state of differentiation; in the B lineage, for example, Ia antigens are not present on pre-B cells, occur at high levels on B cells, and are again absent from plasma cells. Finally, class II MHC gene expression can be modulated by a variety of effectors: γ interferon (IFN-γ) is an up-regulator for macrophages, as is interleukin 4 for B cells, whereas glucocorticoids and α-fetoprotein can be potent down-regulators.

It is important to understand the basis of this elaborate control because aberrant expression of class II MHC molecules may engender or amplify certain of the autoimmune diseases (3). Some hints as to the location of crucial gene promoter elements derive from DNA sequence comparisons. Most suggestive has been the identification of a highly conserved sequence motif in the 5′-flanking region of all MHC class II genes, whether human or murine (4–8). This motif consists of two 14-base conserved segments—the X and Y boxes—spaced by a 17- or 18-base stretch of variable sequence. Although there has been much speculation about how the conserved motif might influence MHC class II gene expression, as yet, no experimental data exist to support any theory.

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Here, we provide evidence that the X and Y boxes play an important role in regulating transcription of a murine class II MHC gene, E^k_α. Mutant genes bearing a clean deletion of either the X or Y box can be transcribed in transgenic mice, but neither efficiently nor accurately. The effect of the Y-box deletion on transcription in IFN-γ-induced macrophages is particularly drastic. In addition, we have been able to detect specific binding of nuclear protein to the X and Y segments.

MATERIALS AND METHODS

Mice. C57BL/6J and SJL/J mice were purchased from Iffa-credo (Les Oncins, France) and Ollac (Bicester, U.K.), respectively. Transgenic mice were produced by injection of E^k_α DNA into (C57BL/6 × SJL)F_2 embryo pronuclei (9). Lines carrying E^k_α were maintained as heterozygotes by back-crossing positive animals to C57BL/6 mice.

Injected DNA. The injected fragments all derive from the A/J strain E^k_α gene carried in the clone λA/J p34.13 (4). The 9-kilobase *Xba* I fragment (position −2170 to position +6800) was subcloned into a pBR322-derived plasmid, and slight modifications were introduced to yield the plasmid pWE32. (*i*) An *Xho* I linker was inserted into the *Acc* I site at position −215. (*ii*) The *Pvu* I site at position +12 was replaced by a *Bam*HI site. The sequence of plasmid pWE32 reads as follows: . . . AGTCTGCAGGATCCGCTTC . . . instead of . . . AGTCTGCGATCGCTTC . . . (*iii*) The *Bam*HI site at position +1581 was eliminated by partial digestion, repair, and ligation.

To construct the ΔX and ΔY mutations, a *Kpn* I–*Sac* I fragment from pWE32 (positions −1320 to +101) was subcloned into M13. X and Y boxes were deleted (individually) by oligonucleotide-directed mutagenesis (10). Mutant phage DNAs were sequenced, and the ΔX21 and ΔY301 mutations were grafted onto pWE32 by substitution of the *Xho* I–*Bam*HI fragment (positions −215 to +12) to yield the plasmids pWEΔX21 and pWEΔY301.

Sucrose-gradient-purified *Bgl* I fragments (8.2 kb) were injected into mouse embryos.

S1 Analysis. The preparation of RNA and its analysis by S1 mapping have been described (11). In most experiments, the probe was a single-stranded *Sac* I–*Xho* I fragment (positions +101 to −215) derived from pWEΔX21, 5′-end labeled at the *Sac* I extremity.

Gel Retardation Assays. Various E^k_α promoter fragments, usually the residue −215 to residue +12 *Xho* I–*Bam*HI segment, were purified by gel electrophoresis followed by electroelution. The 39-base double-stranded oligonucleotides

Abbreviations: MHC, major histocompatibility complex; IFN-γ, γ interferon.

were prepared as follows: complementary strands were synthesized on a Gene Assembler (Pharmacia) using the phosphoramidite method (12); the two strands were 5′ end-labeled using [γ-^{32}P]ATP, combined, denatured for 2 min at 80°C, and annealed 30 min at 20°C; finally, double-stranded oligonucleotides were purified on a 15% polyacrylamide gel. The "Y" oligonucleotide spans positions −74 to −36; i.e., it consists of the Y box plus 13 bases on the 5′ side and 12 bases on the 3′ side. "Y-Rd" includes the same 5′- and 3′-flanking sequences, but the Y box (TTCTGATTGGTTAA) has been replaced by random sequence (ACGTTCTGGCTACA), chosen by flipping two coins. The "X" oligonucleotide spans positions −105 to −68; i.e., it includes the X box and 12 bases on the 5′ side and on the 3′ side. "X-Rd" has the same 5′- and 3′-flanking sequences, but the X box (CCTAGCAACAGATG) has been replaced by random sequence (CATCTGTTGCTAGG).

Nuclear extracts were prepared according to Dignam *et al.* (13), except we routinely used 1 M NaCl to extract nuclear protein. Gel retardation assays were conducted as published (14).

RESULTS

When the immediate 5′-flanking regions of human and mouse class II genes are aligned, a conserved sequence motif becomes apparent (4–8). The consensus is as follows:

$$\underbrace{\text{CCTAG}\overset{\text{CA}}{\text{A}}\text{G}\overset{\text{A}}{\text{T}}\text{GATG}}_{\text{Box X}} - * - \underbrace{\text{T}\overset{\text{T}}{\text{G}}\text{CTGATTGG}\overset{\text{T}}{\text{C}}\text{T}\overset{\text{AA}}{\text{CC}}}_{\text{Box Y}}$$

The distance (denoted by the *) between the X and Y boxes (17 or 18 bases) is also strongly conserved. The placement of X and Y at the 5′ end of E_α^k, a murine class II gene, is diagrammed in Fig. 1 *A* and *B*; the actual sequence of this region is presented in Fig. 1*C*.

X and Y Boxes Are Required for Efficient and Accurate Transcription of E_α^k. To determine whether the X and Y boxes have any regulatory role, we have chosen to study their influence on E_α^k expression in transgenic mice. In previous experiments, we injected this gene into B6 × SJL mouse embryos and developed transgenic lines that harbor the injected DNA stably integrated into the genome (11). (B6 × SJL mice do not transcribe their endogenous E_α genes because of a deletion that removes the promoter region and first exon) (15, 16). The E_α^k transgene was expressed efficiently, accurately, and with tissue and cell type specificity.

For the experiments described here, we created mutant E_α^k genes that carry a clean deletion of the X or the Y box. As a first step, a slightly modified E_α^k, called WE32, was designed; this construct differs from the wild-type gene at only three positions, but greatly facilitates the construction of mutants. Using WE32 as a base, we created the mutants WEΔX21, which bears a clean deletion of the X box (from position −93 to position −80), and WEΔY301, harboring a precise deletion of the Y box (from position −61 to position −48).

The three modified E_α^k genes—WE32, WEΔX21, and WEΔY301—were individually injected into (B6 × SJL)F_2 embryos, always as an 8.2-kilobase *Bgl* I fragment. Multiple transgenic lines were developed for each construct. The number of integrated copies of the injected gene varied among the different lines, fluctuating from 1 or 2 copies to >50.

Expression of the E_α^k Transgene in the Spleen. As a first test of transgene function, we measured E_α RNA expression in the spleen by S1 mapping (Fig. 2). This allowed an estimate of E_α RNA levels as well as an assessment of the accuracy of initiation. To facilitate quantitation in this particular experiment, we used a single probe derived from WEΔX21 that extends from position +101 to position −215.

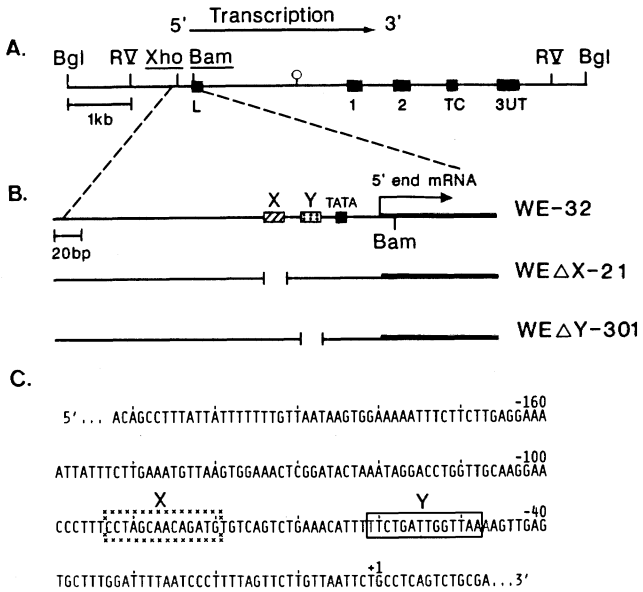

FIG. 1. The E_α^k gene and mutants thereof. (*A*) Diagram of the injected *Bgl* I fragment. Black boxes represent the E_α^k exons as follows: L, leader peptide; 1 and 2, extracellular domains; TC, transmembrane segment and cytoplasmic tail; 3UT, 3′-untranslated region. The open circle denotes a mutated *Bam*HI site; the underlined *Xho* I and *Bam*HI sites were introduced artificially. Bgl, *Bgl* I; RV, *Eco*RV. (*B*) The 5′ end. The mRNA coding region appears as a bold line. (*C*) Sequence of the E_α^k gene 5′ end. The major mRNA start site is at position +1.

The B×A lane illustrates the pattern of protected fragments for a nontransgenic, positive control: the bands at positions +1 and +3 reflect the normal E_α^k RNA initiation sites; the lower bands (arrow) also signify that RNA is initiated at positions +1 and +3, but the fragments protected by these transcripts have been digested internally by S1 at position +12, where the wild-type E_α^k sequence differs from the WEΔX21 probe by a 4-base-pair insertion. The B×S lane confirms that nontransgenic B6 × SJL mice do not express E_α^k RNA.

Two transgenic lines bearing WE32 were studied to verify that the seemingly minor alterations introduced to facilitate the construction of E_α^k mutants were indeed innocuous. As Fig. 2 attests, the slightly modified E_α^k transgene is expressed efficiently and accurately in the spleen. In this instance, we do not observe the bands at position +12 (cf. B×A lane) because WE32 and the WEΔX21 probe are not mismatched at this position.

E_α RNA is expressed in the spleens of all mice transmitting WEΔX21 with the mutant gene bearing a clean deletion of the X box. However, most of this RNA is abnormal. A small percentage of the transcripts are initiated at positions +1 and +3, the normal sites, but by far the majority start at alternative sites scattered throughout the 5′ end. The scatter is even more extensive than is apparent in Fig. 2 since the band labeled Up marks the end of the probe. With other probes, it is possible to detect transcripts that initiate as far upstream as the −1.0- to −1.5-kb region, and this finding is consistent with results of RNA gel blotting experiments (data not shown). There appears to be a rough correlation between levels of mRNA and the number of integrated gene copies.

Mice transmitting ΔY301, the mutant E_α^k that bears a precise deletion of the Y box, exhibit one of two phenotypes. Some—lines 22, 45, and 59—have little or no E_α RNA in the spleen; others—lines 54 and 58—have normal or greater than normal levels of E_α RNA, but again initiation of these

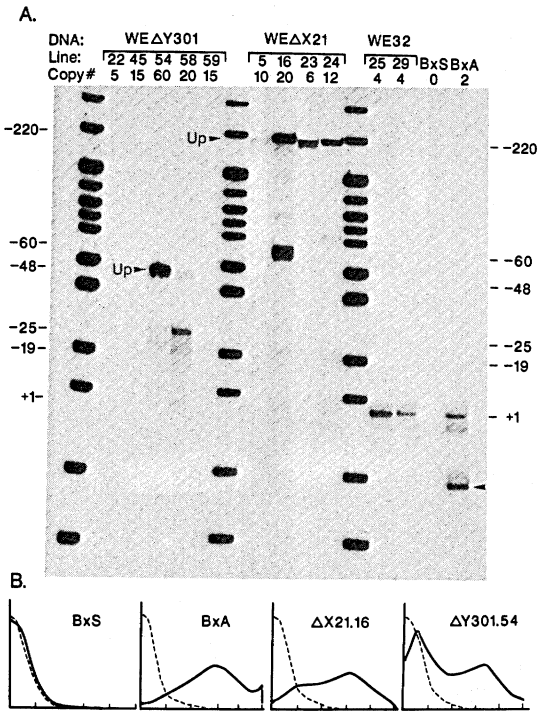

FIG. 2. Transgene expression in the spleen. (*A*) S1 nuclease mapping of E_α transcripts in spleen RNA. The probe was a single-stranded *Sac* I–*Xho* I fragment (residues +101 to −215) from pWEΔX21. Bands labeled Up denote for WEΔX21 all initiation on the 5′ side of residue −215 (the end of the probe) and for WEΔY301 all transcripts initiated on the 5′ side of residue −48 (the point of the ΔY deletion). B×S, (C57BL/6 × SJL) F_2; B×A, (C57BL/10 × A/J)F_1. Copy number was estimated by Southern blotting. (*B*) Flow cytometry of splenic B cells, achieved by gating on surface IgM^+ cells after double-staining with a fluorescein-isothiocyanate-labeled anti-mouse IgM antibody and a phycoerythrin-labeled anti-E reagent (14-4-4). The dotted line in each panel shows cells stained with an irrelevant antibody. WEΔX21-16 and WEΔX301-54 are second generation.

transcripts is aberrant. The spread of transcripts is greater than what is apparent in Fig. 2 since the Up band reflects the position (residue −48) at which WEΔY301 and the probe from WEΔX21 are mismatched. In fact, S1 mapping with other probes and RNA gel blotting provides evidence for transcripts initiating as far on the 5′ side as the −1.0- to −1.5-kb region (data not shown). Note that the two lines that express significant levels of E_α RNA carry many copies (>20 copies) of the transgene.

It was of interest to determine whether the aberrantly initiated transcripts from WEΔX21 and WEΔY301 spleens can be translated to make E_α surface protein. Thus, we isolated splenocytes from various animals, and quantitated E-complex levels on B cells by two-dimensional cytofluorimetry, using a phycoerythrin-labeled anti-E antibody and a fluorescein isothiocyanate-labeled anti-IgM antibody. Fig. 2*B* shows the profiles for control, nontransgenic animals (B×S and B×A). As anticipated, none of the B×S B cells express the E complex, whereas almost all of the B×A IgM^+ cells are also E^+. In other experiments, WE32 transgenics exhibit essentially the same staining pattern as the B×A controls (data not shown). Staining profiles for B cells from WEΔX21.16 spleens show that almost all are E^+, although there seems to be some trailing of cells into the dull-staining region. In contrast, only about half the B cells from the WEΔY301.54 line are surface E^+, although most of the cells that do express the E complex at the cell surface display normal amounts compared with the B×A controls. Qualitatively similar profiles have been observed with other WEΔY301 and WEΔX21 lines.

IFN-γ Inducibility of the E_α^k Transgene. To determine whether the mutated E_α^k transgenes were inducible by IFN-γ, we isolated thioglycollate-primed peritoneal macrophages from various animals, cultured them in the presence or absence of IFN-γ, isolated RNA, and assayed E_α transcripts by S1 mapping (Fig. 3*A*). The B×A panel shows the degree of stimulation for a nontransgenic control: IFN-γ increases the amount of E_α transcript in RNA from peritoneal macrophages 10- to 20-fold, and this level reaches 30–60% of that observed in spleen RNA from the same animal. E_α RNA is induced to a similar degree in the WE32.25 transgenic line (data not shown). In WEΔX21 animals, IFN-γ increases the amount of E_α transcript, whether initiated at promoter-proximal or promoter-distal sites, and the amount in macrophage RNA is again about half that seen for spleen. Note, however, that there are qualitative differences between the spleen and macrophage patterns: for WEΔX21 a strong band at position −55 with spleen RNA is virtually absent with macrophage RNA.

IFN-γ induction of E_α RNA synthesis in WEΔY301 peritoneal macrophages is more complex. In line 58 mice, there is no detectable induction of E_α mRNA. Note (as above) that normal induction would result in E_α RNA levels reaching 30–60% those observed in the spleen. We know that the interferon treatment was effective because the endogenous A_α gene was induced to normal levels (data not shown). In line 54 mice, there is no induction of the E_α transcripts initiated at promoter-proximal sites, but there is an increase in the number of transcripts initiated at promoter-distal sites, although the levels remain well below spleen levels. (Here again, recall that the Up band at position −48 reflects initiation at all sites upstream from that point; this position

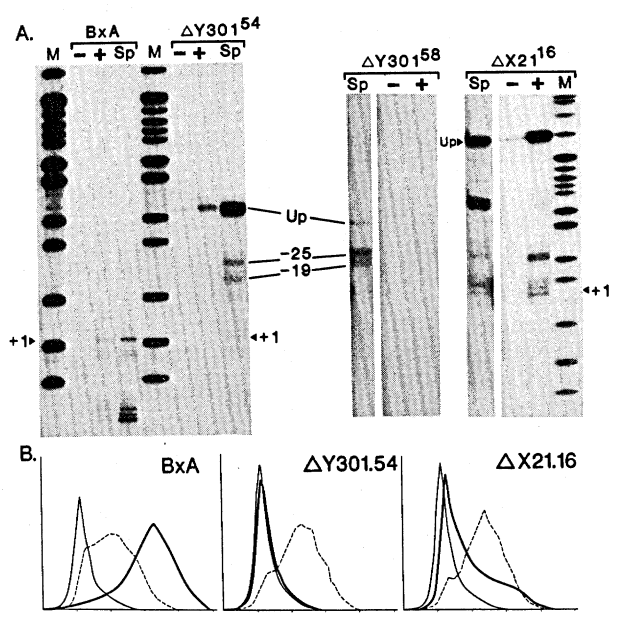

FIG. 3. Transgene expression in macrophages. (*A*) S1 nuclease mapping of E_α transcripts in RNA from spleen (sp) or peritoneal macrophages cultured with IFN-γ (+) or alone (−). The right-most and left-most panels show results from independent experiments. For the former, the probe was a residues +72 to −215 fragment, 5′-end-labeled at residue +72, and, for the latter, it was a residues +101 to −215 fragment, 5′-end-labeled at residue +101. Both probes derive from pWEΔ21. Transgenic mice are second generation offspring. (*B*) Flow cytometry of peritoneal macrophages cultured with (+) or without (−) IFN-γ and then stained with an anti-E antibody, 14-4-4. Dotted lines show staining profiles for IFN-γ-stimulated macrophages stained with an anti-A antibody, 3G2.

marks the point of mismatch between WEΔY301 and the WEΔX21 probe.) Whether or not this distinction between the two WEΔY301 lines is a true difference is not certain since for line 58 mice there are so few transcripts initiated at promoter-distal sites. In any case, we have seen no induction of the promoter-proximal transcripts around positions −25 and −19 for either line in multiple experiments.

Thus, the Y box seems particularly critical for expression of E_α transcripts in IFN-γ-induced macrophages. This finding has been confirmed by cytofluorimetric quantitation of E complex on the surface of peritoneal macrophages. As seen in Fig. 3*B*, there is no measurable $E_\alpha E_\beta$ protein on stimulated WEΔY301 macrophages, though a clear induction is discernible on cells from B×A mice (and from WE32 animals, data not shown). WEΔX21 mice do have E complex on IFN-γ-induced macrophages, but the level is somewhat reduced, probably because many of the RNAs initiate far upstream.

Nuclear Proteins Bind Specifically to the X and Y Boxes. Specific binding of nuclear proteins to the 5′-flanking region of E_α^k has been studied with the gel retardation assay. This technique (17) depends on the relative binding affinity of a given protein for a ^{32}P-labeled DNA fragment versus unlabeled nonspecific competitor DNA. If the protein binds specifically to the labeled DNA, migration of the resulting DNA–protein complex in a neutral polyacrylamide gel will be retarded with respect to migration of the naked DNA fragment.

NF-Y, a Y box-binding protein. Fig. 4*A* shows a gel retardation assay using the ^{32}P-labeled *Xho* I–*Bam*HI fragment from the 5′-flanking region of E_α^k (positions −215 to +12, see Fig. 1), various quantities of poly[d(I-C)] competitor, and a 1 M NaCl nuclear extract from the B lymphoma M12. As the amount of unlabeled competitor DNA increases, a distinct band becomes apparent (arrow). This band reflects the binding of a protein (hereafter referred to as NF-Y) to the Y box. The evidence for this conclusion is 3-fold. (*i*) Gel retardation assays using a panel of mutant E_α promoter fragments show a perfect correlation between the presence of the Y box on a given fragment and its ability to bind NF-Y (Fig. 4*B*). In particular, a clean deletion of this 14-base segment completely abrogates binding (compare WT and 301 panels in Fig. 4*A*). On the other hand, removal of the TATA box (Δ26), X box (Δ21), or GTGGAAA motifs (Δ6, BR) does not reduce binding of NF-Y. (*ii*) DNase I footprinting, methylation interference mapping, and point-mutational analyses have delineated contact sites for NF-Y within the Y box (27). (*iii*) NF-Y can be bound by a 39-mer (the Y oligonucleotide) centered on the Y box. The prominent retarded band in Fig. 5 does reflect binding of what we have termed NF-Y because this band is not observed in assays that employ the Y-Rd oligonucleotide, which contains the same flanking nucleotides but has random sequence replacing the Y box. Furthermore unlabeled Y oligonucleotide, but not unlabeled Y-Rd oligonucleotide, competes for the binding of NF-Y to the *Xho* I–*Bam*HI E_α^k promoter fragment and unlabeled wild-type E_α^k promoter fragment, but not the same fragment from Δ301, competes for specific protein binding to the Y oligonucleotide (data not shown).

X box-binding protein. Gel retardation experiments with the various mutant fragments depicted in Fig. 4*B* (in particular with the Δ21 fragment) provided no evidence for proteins that bind specifically to the X box. But the better visualization of NF-Y afforded by using a short 39-base fragment prompted us to search for X-binding proteins using an oligonucleotide. Thus, we made the 38-base X oligonucleotide, composed of the X box and 12 bases on each side, and the X-Rd oligonucleotide, which has the same 5′- and 3′-flanking bases but random sequence replacing the X box. As seen in Fig. 5, we detect three faint bands (arrowed) that are specific to the X box. All the other bands are present in

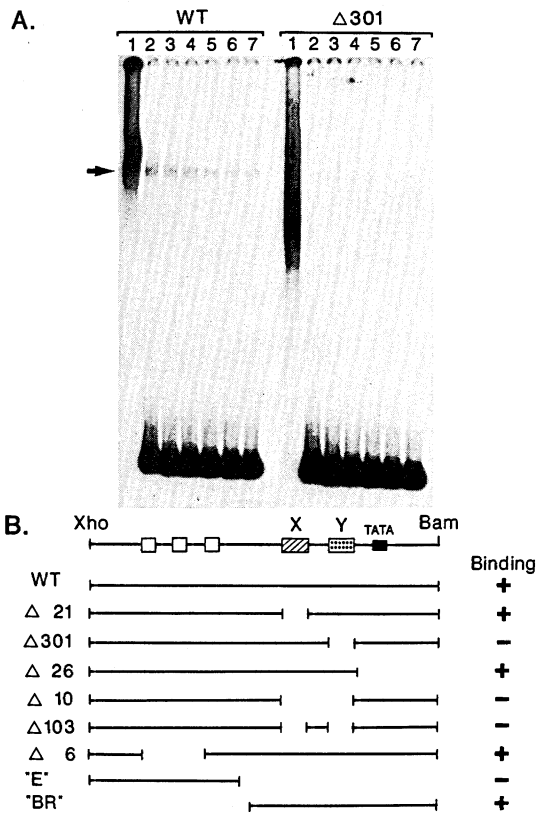

FIG. 4. Gel retardation assays on wild-type and mutant promoter fragments. (*A*) A ^{32}P-labeled fragment (residues −215 to +12) from wild-type E_α^k (WT) or from a Y-box deletion mutant (Δ301, see below) were mixed with increasing amounts of poly[d(I-C)] and 1–2 μg of nuclear extract from the B lymphoma M12. The position of a stable DNA–protein complex is indicated with an arrow; the strong bottom band represents free DNA. Amounts of poly[d(I-C)] for lanes 1–7 were 0, 0.3, 0.6, 0.9, 1.2, 1.5, and 2 μg. (*B*) Effect of promoter deletions. Symbols are defined in the legend to Fig. 1. Deletions were made by BAL-31 or restriction enzyme digestion or by oligonucleotide-directed or spontaneous mutagenesis. Their positions are: Δ21, residues −93 to −80; Δ301, residues −61 to −48; Δ26, residues −40 to +1; Δ10, residues −93 to −48; Δ103, residues −43 to −80 and residues −61 to −48; Δ6, residues −186 to −142; E, residues −129 to +12; BR, residues +215 to −113.

the X-Rd control and thus reflect nonspecific binding or attachment of protein to the flanking sequences. Also, most of these extraneous bands disappear at quite low poly[d(I-C)] concentrations. Unfortunately, the bands that correlate with

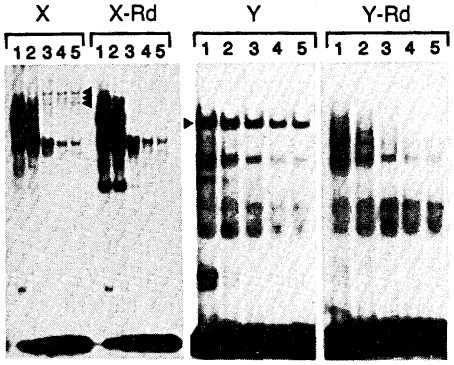

FIG. 5. Specific binding to oligonucleotides. The labeled DNAs were the double-stranded oligonucleotides Y, Y-Rd, X, and X-Rd. Poly[d(I-C)] concentration was 0, 50, 100, 200, or 300 ng per lane for Y and Y-Rd in lanes 1–5, respectively; and was 50, 100, 300, 600, and 800 ng per lane for X and X-Rd in lanes 1–5, respectively.

the X box are so weak that we have been unable to verify the protein binding site by methylation interference or DNase footprinting.

DISCUSSION

It has been noted repeatedly that the MHC class II genes harbor a conserved sequence motif in the 5′-flanking region—the X and Y boxes (4–8). Here, we provide evidence that these elements play a role in regulating transcription by assessing the effect of their deletion on E_α RNA synthesis in transgenic mice. Deletion of either the X or Y box has two profound effects: first, the efficiency of transcription is reduced; second, RNA initiation is no longer accurate. The first point is based on the observation that wild-type levels of E_α RNA only occur when many copies of the transgene are present. Thus, we surmise that high copy number compensates for weak promoters. Some of the variability may be due to position effects, but we do not think this is an important influence because the wild-type E_α gene seems to be rather oblivious to position effects in the four independent lines we have tested.

The ΔX and ΔY mutants have superficially similar phenotypes: both show reduced efficiency and abnormal initiation of E_α transcription; furthermore, the tissue specificity of E_α RNA synthesis is maintained, in the sense that there are high levels of RNA in the spleen but no detectable transcripts in heart and brain (data not shown). One might argue, then, that the altered phenotype could result completely or partially from a perturbation of promoter structure due to the loss of 14 nucleotides rather than from the particular deletion itself. While this work was in progress, Takahashi *et al.* (18) demonstrated that the distance between promoter elements can affect their function, probably due to stereospecific alignment of proteins along the DNA. Thus, our results should be confirmed with X and Y box point mutations, rather than deletions. In the meantime, we emphasize that important distinctions do exist between the ΔX and ΔY phenotypes: the distribution of aberrant start sites differs, the sites for WEΔY301 being much more clustered; in addition, the Y box, but not the X box, deletion essentially abolishes E_α transcription in IFN-γ-treated macrophages. This characteristic also extends to E_α expression in mature, uninduced macrophages, which appear largely E^- in spleen or thymus sections from ΔY301-54 mice (data not shown; W. Van Ewyk, personal communication).

As we discuss at length elsewhere (27), the Y box TGCTGATTGGCTCC actually contains a CCAAT sequence in reverse. CCAAT has been shown to be an important component of several promoters and can operate in reverse orientation as ATTGG (for review, see ref. 19). CCAAT deletions or mutations have been shown to affect basal levels of transcription in tissue culture cells (ref. 20 and references therein) and to be capable of provoking aberrant initiation of mRNA (21). But the CCAAT sequence and adjacent nucleotides may also have a more complicated function, modulating transcript levels during differentiation (22, 23) or mediating induction by certain effectors (21, 24, 25). Not surprisingly, then, the CCAAT/ATTGG sequence specifically binds a nuclear protein or proteins of broad cell-type distribution (for references, see ref. 19). It is of interest to reflect on the Y box in this context. Our data show that the Y box influences basal levels of transcription as well as the accuracy of mRNA initiation. Perhaps of more consequence, our results provide support for the contention that the CCAAT box and adjacent sequences can serve a more sophisticated role: the Y box, which harbors a CCAAT sequence, is indispensable for IFN-γ induction of E_α transcripts initiated in its vicinity. We have detected a protein that binds specifically to the Y box, but we do not know how, or even if, this protein mediates E_α expression in macrophages. Since NF-Y is a CCAAT box-binding protein and has a ubiquitous tissue distribution, it is most likely that at least one other protein is involved.

Our evidence for the importance of the MHC class II conserved motif relies largely on studies of transcription in transgenic mice. We chose transgenic mice, rather than cells in culture, as an assay system because MHC class II genes are subject to an interactive network of regulatory influences, and it would be difficult, if not impossible, to reproduce this complexity with cultured cells (a problem already documented for other genes, see ref. 26). In addition, we had hoped to, and apparently have been able to, create new mouse strains that harbor class II genes expressed in only certain compartments of the immune system.

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