Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4254–4258, June 1987
Immunology

Upstream DNA sequences required for tissue-specific expression of the *HLA-DRα* gene

(octamer/class II box/protein–DNA complexes/electrophoretic mobility shift/gene regulation)

PAULA A. SHERMAN*, PATRICIA V. BASTA†, AND JENNY P.-Y. TING*†

*Lineberger Cancer Research Center and †Department of Microbiology/Immunology, University of North Carolina, Chapel Hill, NC 27514

Communicated by Mary Ellen Jones, March 16, 1987 (received for review December 3, 1986)

ABSTRACT **We have used *in vitro* deletion mutagenesis in combination with DNA transfection to search for cis-acting regulatory elements involved in the tissue-specific expression of a human class II major histocompatibility complex gene. A 140-base-pair 5′ flanking fragment that contains the class II box consensus sequences and an octamer sequence (ATTTGCAT) confers tissue specificity on the promoter of the *HLA-DRα* gene. Recombinant DNA plasmids containing this *DRα* gene segment fused to the coding sequence of the bacterial chloramphenicol acetyltransferase gene are expressed at higher levels in human B-cell lines than in human T-cell lines. We have demonstrated that the most 5′ of the class II boxes is essential for tissue-specific *DRα* promoter function. In addition, using an electrophoretic mobility shift assay to identify DNA binding proteins, we have detected binding of nuclear proteins to DNA probes containing the class II boxes and the octamer sequence. A protein that binds to the octamer is present at higher levels in nuclear extracts of B-cell lines than in other cell lines examined. This protein may be important for the tissue-specific expression of the *HLA-DRα* gene.**

Class II major histocompatibility complex (MHC) antigens are cell-surface glycoproteins that function in immune cell–cell recognition and interaction. These molecules control the level of immune response to certain antigens through their role in the presentation of antigen to class II-restricted, antigen-specific T cells (1). There are at least three human class II antigens, designated DR, DP, and DQ. All class II molecules are composed of two noncovalently associated subunits. The heavy (α) chain is relatively nonpolymorphic, and the light (β) chain is highly polymorphic.

The genes coding for the class II MHC antigens are transcribed in a tissue-specific manner (2). Class II antigens are found primarily on certain cells of the immune system, including B lymphocytes, macrophages, and some activated T cells (3). Class II gene expression is differentially regulated in different cell types and is responsive to multiple regulators (4). For example, class II antigen expression on B cells is modulated by B-cell growth factor (5), whereas that on macrophages is modulated by γ-interferon, α-fetoprotein, and prostaglandin E (4). γ-Interferon can enhance or induce the expression of class II genes in many different cell types, including some nonlymphoid cells (6).

A few studies have employed gene transfer experiments and sequence homology searches to identify cis-acting transcriptional regulatory elements in class II genes. Gene transfer experiments have demonstrated that a B-lymphocyte-specific enhancer element is located in a 2.0-kilobase (kb) 5′ flanking fragment of the mouse E^d_β gene (7). Our laboratory has identified a region in the 5′ flanking sequence of the *HLA-DRα* gene that is important for γ-interferon induction of class II antigens in human glioma (8) and melanoma (unpublished results) cell lines. In a similar study, another laboratory has characterized DNA elements that are involved in the γ-interferon induction of the *HLA-DQ2β* gene in human fibroblasts (9). A distinct consensus sequence for α-interferon induction is located further upstream from the cap site than the γ-interferon response sequence in HLA class II genes (10).

Sequence homology searches have revealed that human and murine class II α- and β-chain genes possess two short conserved sequences positioned at a distance of 100–150 base pairs (bp) on the 5′ side of the ATG start codon for translation (11). These consensus sequences, referred to as class II boxes, are possibly involved in the regulation of class II gene expression. In addition, an octanucleotide sequence that is found ≈70 bp upstream from the site of transcriptional initiation in all immunoglobulin light- and heavy-chain genes is also found ≈40 bp upstream from the cap site in the *HLA-DRα* gene (12). This octamer sequence is required for tissue-specific expression of immunoglobulin genes (13–15).

In this report, we investigate the cis-acting DNA sequences that control tissue-specific expression of class II genes. To do this, we have transfected a set of recombinant plasmids containing 5′ promoter deletions of the *HLA-DRα* gene fused to a bacterial indicator gene into human B- and T-cell lines. We then assayed extracts of transfected cells for *DRα*-controlled transient expression of the indicator gene. We have also used an electrophoretic mobility shift assay to detect specific binding of nuclear proteins to DNA fragments containing the class II boxes and the octamer.

MATERIALS AND METHODS

Plasmids. pSV2CAT is identical to the plasmid described by Gorman *et al.* (16). DNA constructs containing 5′ flanking sequences of the *HLA-DRα* gene fused to the bacterial chloramphenicol acetyltransferase (CAT) gene are described in detail in ref. 8. In brief, pD164-2 is the parental plasmid containing the CAT gene but no *DRα* sequences; pDR1000 contains ≈1000 bp of DNA 5′ to the cap site, the cap site, and 30 bp 3′ to the cap site of the *DRα* gene; and pDR300 is identical to pDR1000 but contains ≈300 bp of DNA 5′ to the cap site. Additional deletion mutants were prepared as follows: pDRA1000, which contains 1000 bp of DRα 5′ flanking sequences (described in ref. 8), was linearized with *Xba* I; this was followed by exonuclease III (Stratagene, San Diego, CA) digestion for 1.5–3.0 min at 26°C. Single-stranded DNA was removed with mung bean nuclease (Stratagene); this was followed by digestion with *Cla* I, treatment with Klenow enzyme to form blunt ends, and religation. The deletion mutants were characterized by dideoxy DNA sequence determination directly from the double-stranded plasmid DNA (17), using a 20-base single-stranded oligonu-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAT, chloramphenicol acetyltransferase; MHC, major histocompatibility complex.

cleotide (prepared by C. Hutchison, University of North Carolina, Chapel Hill) complementary to a 5′ CAT coding sequence (18) as a primer for reverse transcriptase. The 5′ deletion mutants designated 5′ Δ-109, 5′ Δ-91, and 5′ Δ-53 contain *DRα* sequences extending from positions 109, 91, and 53 bp upstream from the cap site to +30 bp (see Fig. 1).

Cell Lines. Raji is a human Epstein–Barr virus (EBV)-positive Burkitt lymphoma cell line; Ramos RA 1 is a human EBV-negative Burkitt lymphoma cell line; 721.174 is a human B-lymphoblastoid cell line (kindly provided by Robert DeMars, University of Wisconsin, Madison); HSB and H9 are human T-leukemia cell lines; and HeLa is a human cervical carcinoma cell line.

Transfection and CAT Assay. Suspension cultures of human lymphoblastoid cell lines were transfected using DEAE-dextran (19). Cells (10^7) were suspended in 2 ml of a transfection cocktail containing 10 μg of plasmid DNA and 500 μg of DEAE-dextran (Pharmacia, average M_r 500,000). After 1 hr at 37°C, the cells were washed twice with serum-free medium and resuspended in 10 ml of complete medium. Approximately 48 hr following transfection, cells were harvested by centrifugation, washed once with phosphate-buffered saline, and resuspended at 50 μl per 10^6 cells in 0.25 M Tris·HCl (pH 7.6). Cells were lysed by several freeze–thaw cycles or by sonication, cellular debris was removed by centrifugation, and the resulting extracts were assayed for CAT activity as described (16). Reaction mixtures contained 25 μl of cellular extract, 0.175 M Tris·HCl (pH 7.6), 1 mM acetyl-CoA, and 0.1 μCi (1 Ci = 37 GBq) of [^{14}C]chloramphenicol (New England Nuclear; specific activity, 55 mCi/mmol) in a total volume of 180 μl. In general, the incubation time was several hours.

Electrophoretic Mobility Shift Assay. This procedure is described in refs. 20–22 and in modified form in ref. 23. Nuclear extracts employed in this assay were prepared according to the protocol of Dignam *et al.* (24) and contained 2–6 mg of protein per ml as assayed by the Bradford dye-binding method (25). Binding reactions contained 25 mM Hepes (pH 7.9), 2.5 mM $MgCl_2$, 25 mM NaCl, 4 μg of poly(dI-dC)·poly(dI-dC) (Pharmacia), 6 μg of nuclear extract protein, and typically 100,000 cpm (0.5 ng) of end-labeled DNA fragment in a total volume of 20 μl. After a 30-min incubation at room temperature, the samples were electrophoresed through a low ionic strength 8% polyacrylamide gel (acryl:bis weight ratio of 29:1) containing 6.7 mM Tris·HCl (pH 7.5), 3.3 mM sodium acetate, and 1 mM Na_2EDTA. The gel was preelectrophoresed for 2 hr at 20 mA and electrophoresed for 2–3 hr at the same current with buffer recirculation. The wet gel was autoradiographed at −70°C with intensifying screens. Two synthetic double-stranded oligonucleotides (prepared by C. Hutchison, University of North Carolina, Chapel Hill) were used as probes in the binding reactions (see Fig. 1). These DNA fragments were end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase (Bethesda Research Laboratories) (26). Labeled fragments were separated from unincorporated radionucleotide by passage over a NENSORB 20 nucleic acid purification cartridge (New England Nuclear).

Methylation Interference Analysis. This procedure was performed exactly as described in ref. 27, except that DNA was isolated from mobility shift gels by overnight agitation of the crushed gel slices in 0.5 M ammonium acetate. Products of the piperidine cleavage reactions were analyzed by electrophoresis through a 12% polyacrylamide/8 M urea sequencing gel.

RESULTS

A 140-bp 5′ Flanking Region Confers Tissue Specificity on the Promoter of the *HLA-DRα* Gene. The 5′ flanking regions of many genes contain important transcriptional regulatory elements (28). For this reason, we initiated our search for DNA sequences involved in the tissue-specific expression of the *HLA-DRα* gene with a 1000-bp 5′ flanking fragment. We fused this DNA fragment to the CAT gene and transfected the resulting recombinant into human B- and T-lymphoblastoid cell lines. Although a detectable level of CAT activity was observed in T cells, much higher levels were consistently observed in B cells, in accord with the tissue-specific expression of the *DRAα* gene by these cell types (Fig. 2, lanes 3 and 7). A deletion mutant containing 300 bp of 5′ flanking sequence yielded similar results (Fig. 2, lanes 4 and 8), except that CAT activity was greater in B cells transfected with pDR300 than with pDR1000. This latter observation suggests the presence of a negative regulatory element in the additional 700 bp of DNA contained in pDR1000. Transfection results similar to those for T cells were observed for HeLa cells (data not shown). Quantitation of plasmid DNA in Hirt supernatants (29) of transfected cells confirmed that the difference in CAT activity in B and T cells was not due to a simple difference in transfection efficiency of the two cell types.

To more precisely define the location of DNA sequences involved in the tissue-specific expression of the *HLA-DRα* gene, we prepared a progressive series of deletion mutants using exonuclease III. Expression of CAT in B cells was still observed with a mutant (Fig. 1, 5′ Δ-109) containing only 140 bp of *DRα* sequence (Fig. 3, lane 5). This DNA fragment contains the class II boxes and the immunoglobulin octamer motif. The decrease in promoter activity observed with this

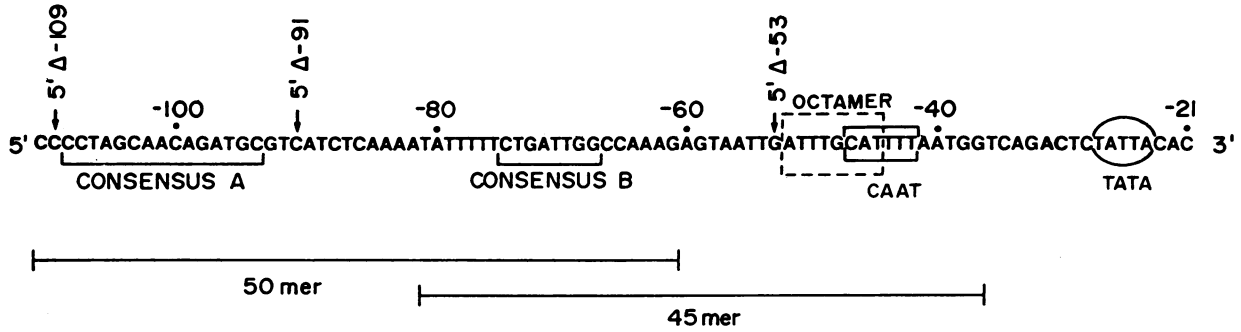

FIG. 1. Regulatory elements in the promoter region of the *HLA-DRα* gene. Nucleotides are numbered relative to the cap site. Potential cis-acting transcriptional regulatory elements include the class II boxes (designated consensus A and consensus B), the octamer motif ATTTGCAT, a CAAT-type sequence, and a "TATA box." The "CAAT box" specified in the figure is not in good agreement with the consensus sequence for CAAT boxes. A perfect "CCAAT box" is located on the opposite strand of the consensus B sequence. The 5′ deletion mutants used in transfection analyses contained *DRα* sequences extending from positions indicated by arrows to +30 bp. Locations of 45-bp and 50-bp oligonucleotides used as probes in protein–DNA-binding assays are shown below the sequence.

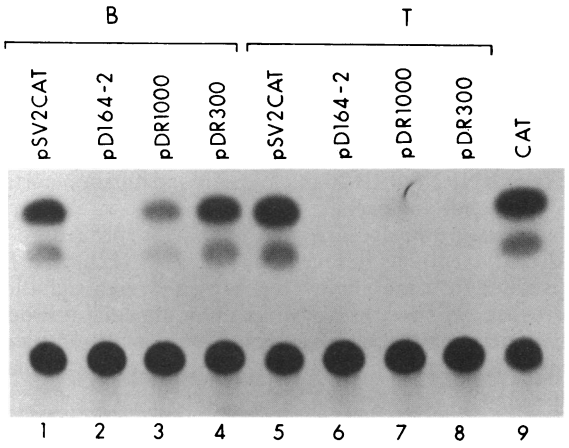

FIG. 2. Transfection of human B cells (B) and T cells (T) with *DRα*–CAT recombinants. Raji and H9 cells were transfected using DEAE-dextran. Extracts of transfected cells were analyzed for CAT activity by thin-layer chromatography and autoradiography. The autoradiograph shows relative amounts of acetylated chloramphenicol reaction products. pSV2CAT was used as a positive control to show that Raji and H9 cells transfect with approximately equal efficiency (lanes 1 and 5). The negative control was pD164-2, which contains CAT coding sequences but no *DRα* sequences (lanes 2 and 6) (8). pDR1000 and pDR300 contain ≈1000 bp and ≈300 bp, respectively, of DRα 5′ flanking sequence (8). Lane 9, reaction products obtained using a commercial preparation of bacterial CAT.

deletion mutant is probably due to the removal of some sequences important for *DRα* expression that are located 5′ of the class II boxes. Deletion of the class II box most 5′ to the cap site (deletion mutant 5′ Δ-91) abolished the activity of the *DRα* promoter in our assay system, suggesting that this DNA sequence is an essential cis-acting transcriptional regulatory element in the *DRα* gene (Fig. 3, lane 6). In a predictable fashion, deletion of both class II boxes (deletion mutant 5′ Δ-53) also destroyed *DRα* promoter activity (Fig. 3, lane 7).

A Protein in B-Cell Nuclear Extracts Binds to the *HLA-DRα* Gene Promoter Region. The high degree of sequence and positional conservation of the class II boxes, in combination with their functional requirement in *DRα* gene expression (as indicated by our transfection data), suggests that these DNA sequences may interact with sequence-specific transcription factors. We were also interested in looking for specific

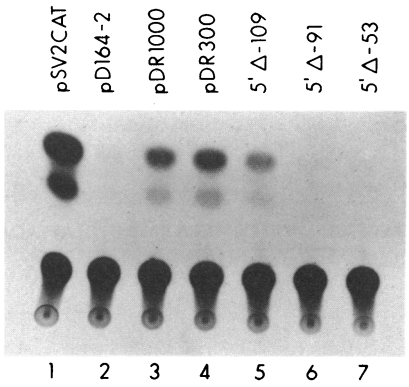

FIG. 3. Transfection of human B cells with deletion mutants. Autoradiograph of CAT assay on extracts of Raji cells transfected with a progressive series of DRα deletion mutants. Lane 1, pSV2-CAT used as a positive control; lane 2, negative control; lane 3, plasmid contains 1000 bp of DRα 5′ flanking sequence; lane 4, plasmid contains 300 bp of DRα 5′ flanking sequence; lanes 5–7, deletion mutants as shown in Fig. 1.

protein interactions with the octamer motif located in the *DRα* promoter, due to recent reports of nuclear factors binding to this sequence in immunoglobulin genes (23, 27, 30). We were particularly interested in nuclear factors specific to B cells, since these factors could be involved in the tissue-specific expression of the *DRα* gene. To this end, we have used an electrophoretic mobility shift assay to identify DNA-binding proteins. This assay is based on the retarded migration of protein–DNA complexes relative to free DNA during gel electrophoresis. Since the use of small DNA fragments as probes enhances the sensitivity of the assay (23), we began our studies with a 45-bp synthetic oligonucleotide that contains one of the class II boxes (consensus B) and the octamer motif (Fig. 1). When we used the labeled 45-bp oligonucleotide as a probe in the mobility shift assay, we observed that several DNA–protein complexes were formed (Fig. 4). All nuclear extracts tested displayed a band, B1, which is most likely generated by a previously described ubiquitous octamer-specific DNA-binding protein (23). Of primary interest is a band, B2, migrating faster than B1, which is most intense in binding reactions containing nuclear extracts of B-cell lines. This complex is much less abundant, if present at all, in binding reactions containing nuclear extracts of T-cell lines and HeLa cells.

The B-Cell Nuclear Factor Is an Octamer-Binding Protein. To establish the specificity and the site of the DNA–protein interaction in B2, a binding competition analysis was performed. The 45-bp oligonucleotide containing the consensus B sequence and the octamer, a 50-bp oligonucleotide containing consensus A and consensus B sequences (see Fig. 1), and a 27-bp oligonucleotide of unrelated sequence were used as unlabeled competitors in the mobility shift assay. The results of this experiment are shown in Fig. 5.

The 45-bp oligonucleotide that contains the octamer sequence competes in the formation of B1 and B2 (lane 4). In contrast, the 50-bp class II box-containing oligonucleotide and the unrelated 27-bp oligonucleotide do not affect the formation of either complex. Hence, only an octamer-containing competitor has an effect on the formation of protein complexes with a DNA probe that contains the octamer sequence and one class II box. These data strongly suggest that the factors involved in the formation of B1 and B2 are octamer-specific proteins. Further support for this conclusion was obtained by employing the 50-bp oligonucleotide that contains both class II boxes as a probe in the

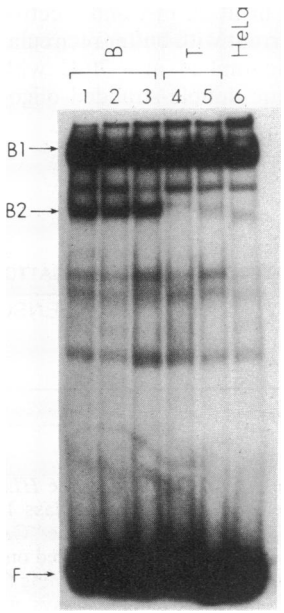

FIG. 4. Electrophoretic mobility shift assays of nuclear extracts from human B, T, and HeLa cells. A 45-bp oligonucleotide containing one of the class II boxes and the octamer motif (see Fig. 1) was used as a probe in the binding assay. Protein–DNA complexes were electrophoresed through an 8% polyacrylamide gel. The nuclear extracts were prepared from the following cell lines: Raji (lane 1), 721.174 (lane 2), Ramos RA 1 (lane 3), HSB (lane 4), H9 (lane 5), and HeLa (lane 6). B1, position of a complex generated by a nuclear factor(s) present in all cell types examined; B2, a complex generated by a factor(s) most abundant in B cells; F, free probe.

binding assay. We do not detect any complexes exhibiting B-cell specificity with this probe (Fig. 5, lane 9). We do detect one complex that migrates with a mobility similar to B1 in all of the extracts tested. Competition analysis suggests that this complex results from a specific interaction between the class II boxes and a nuclear protein; the 45-bp oligonucleotide and the 50-bp oligonucleotide compete in the formation of this complex (Fig. 5, lanes 10 and 11), whereas the unrelated 27-bp oligonucleotide does not (Fig. 5, lane 12).

As a final proof that the nuclear factors involved in the formation of B1 and B2 are octamer-binding proteins, we performed a methylation interference analysis (27). Each strand of the 45-bp oligonucleotide was labeled at the 5′ end using T4 polynucleotide kinase. The DNA was then partially methylated on guanines with dimethyl sulfate and used as a probe in the electrophoretic mobility shift assays as described above. The DNA in complexes B1 and B2 (formed in binding reactions containing nuclear extracts of Raji cells) and the free DNA were isolated from the mobility shift gel, cleaved with piperidine, and electrophoresed through a denaturing polyacrylamide sequencing gel. If any of the methyl groups introduced by dimethyl sulfate interfered with the binding of a specific protein, then that molecule of DNA would be selectively missing in the complex band and subsequently in the corresponding guanine ladder. The results of this experiment are shown in Fig. 6. In the 45-bp oligonucleotide, methylation of the two guanine residues in the octamer sequence interferes with DNA–protein interactions in B1 and B2; no additional guanine residues appear to be involved in complex formation. We conclude that the nuclear factors in B1 and B2 are octamer-binding proteins, and their binding sites are indistinguishable.

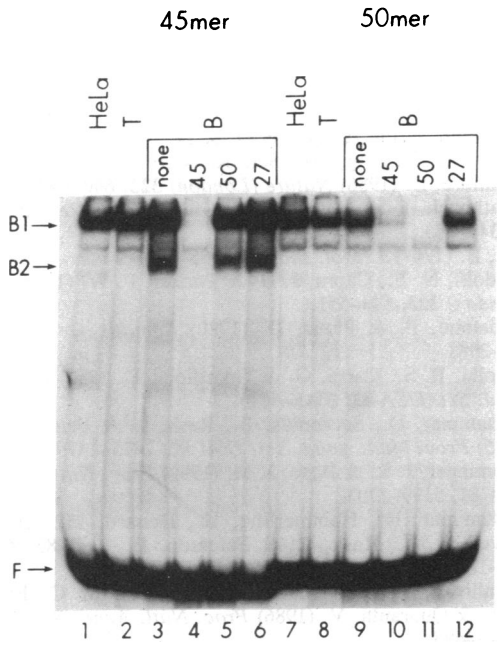

FIG. 5. Binding competition analysis. As indicated above the lanes, various unlabeled DNA fragments were used as competitors in the electrophoretic mobility shift assay. These competitors were added in the following amounts to binding reaction mixtures containing as a probe ≈1 ng of labeled 45-bp oligonucleotide (lanes 1–6) or ≈1 ng of labeled 50-bp oligonucleotide (lanes 7–12): 45 bp, 100 ng; 50 bp, 120 ng; 27 bp, unrelated sequence, 150 ng. Lanes 2 and 8, nuclear extracts of HSB cells. Lanes 3–6 and lanes 9–12, nuclear extracts of Raji cells. B1 and B2, positions of complexes as described in the legend to Fig. 4.

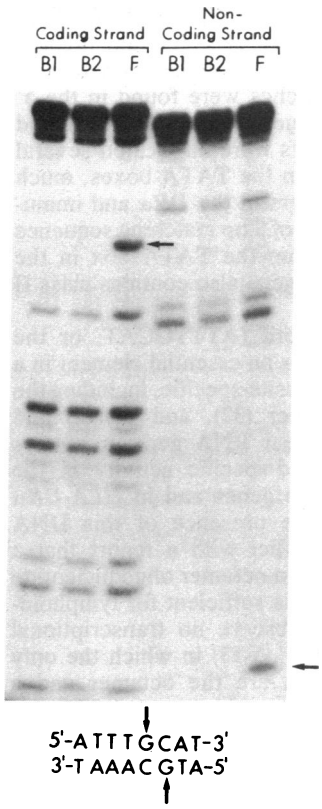

FIG. 6. Methylation interference analysis of proteins binding to the 45-bp oligonucleotide. DNA was 5′ end-labeled on either the coding or the noncoding strand, partially methylated with dimethyl sulfate, and incubated in binding reaction mixtures with a Raji cell nuclear extract. DNA from complexes B1 and B2 (as described in the legend to Fig. 4) and free DNA (F) were isolated from mobility shift gels, cleaved with piperidine, and electrophoresed through a 12% polyacrylamide sequencing gel. The arrows indicate the locations of two guanine residues at which methylation specifically interferes with protein binding. As shown below the autoradiograph, both of these guanine residues are located in the octamer sequence.

DISCUSSION

DNA transfection is a useful functional assay for cis-acting elements involved in the transcriptional regulation of genes. Our gene transfer results indicate that DNA sequence information sufficient for B-cell-specific expression of the *HLA-DRα* gene is contained in a 140-bp promoter segment. This DNA fragment includes class II boxes and an octamer sequence, ATTTGCAT. This finding does not exclude the possibility that additional cis-acting elements conferring tissue specificity (such as an enhancer) are located elsewhere in the *DRα* gene.

Using an electrophoretic mobility shift assay to identify sequence-specific DNA-binding proteins, we have detected a factor in nuclear extracts of human B-cell lines that binds to an octamer-containing fragment of the *DRα* gene. Nuclear extracts of human T-cell lines and HeLa cells contain none or very little of this factor. These findings are very similar, if not identical, to reports of lymphoid-specific protein binding to the octamer sequence in immunoglobulin genes. Staudt *et al.* (27) describe a nuclear protein, NF-A2, that binds to the octamer sequence in the immunoglobulin κ-chain promoter and that is found only in nuclear extracts of lymphoid cell lines. Landolfi *et al.* (30) report a similar lymphoid-specific protein that binds to the octamer sequence (inverted orientation, ATGCAAAT) in the immunoglobulin heavy-chain promoter. In contrast with our results, both of these groups do detect the factor in nuclear extracts of T-cell lines. Both reports suggest that the lymphoid-specific octamer-binding protein accounts for the lymphoid-specific activity of immunoglobulin promoters. The protein that we describe may in a similar fashion be involved in the tissue-specific expression of the *HLA-DRα* gene and may be the hypothetical "trans-acting factor" that regulates class II gene expression in B cells (31, 32).

If the octamer is an essential regulatory element in the *DRα* gene promoter, one might predict that this sequence would be found in a similar location in other class II genes. However,

when we searched for an octamer motif (ATTTGCAT or ATGCAAAT) in all human and murine class II α- and β-chain genes sequenced to date, no perfect homologies were detected. Seven of eight base-pair matches were found in the 5′ flanking regions of a *DR*β pseudogene (33), *DC-3*β (34), and A^d_β (35). These octamer-like motifs were all located several hundred base pairs upstream from the TATA boxes, much further upstream than the octamers in the *DR*α and immunoglobulin promoters. A similar 7 of 8 bp matching sequence is located $\approx$260 bp upstream from the TATA box in the human invariant chain gene; this gene also contains class II boxes (36).

Paradoxically, the octamer motif (ATTTGCAT, or the inverse sequence, ATGCAAAT) is an essential element in a variety of promoters that are not tissue-specific, including the human histone 2B gene promoter (37), and human and *Xenopus* U1 and U2 small nuclear RNA gene promoters (38–41). Therefore, the lymphoid-specific activity of the octamer motif in immunoglobulin genes and in *HLA-DR*α does not result from the simple presence of this DNA sequence in a promoter. In conflict with a report that a synthetic promoter consisting of an octamer oligonucleotide placed upstream of a TATA box is sufficient for lymphoid-specific transcription (27), we observe no transcriptional activity with a deletion mutant (5′ Δ-53) in which the only recognizable regulatory elements are the octamer and a TATA box. Lymphoid specificity could nonetheless result from the interaction of a lymphoid-specific protein with the octamer in the promoters of the immunoglobulin and *DR*α genes. In the histone 2B and small nuclear RNA genes, factors distinct from this lymphoid-specific protein might preferentially interact with the octamer to activate transcription. Such differential binding of proteins to the same DNA sequence is somewhat difficult to comprehend but may involve interactions with additional regulatory factors. For example, in the *HLA-DR*α gene promoter, factors binding to the class II boxes could influence binding to the octamer sequence. This hypothesis is supported by our transfection data that indicate that at least the most 5′ of the class II boxes is required for *DR*α gene promoter function. A detailed understanding of how the octamer element functions as a transcriptional regulatory element may therefore require the identification and purification of multiple, distinct, octamer-specific DNA-binding proteins.

We observe one predominant DNA–protein complex when we use a synthetic oligonucleotide containing both class II boxes as a probe in the electrophoretic mobility shift assay. No tissue-specific complexes are detected with this probe. Further studies will be required to determine the relationship, if any, of protein(s) that bind to the class II boxes to proteins that bind to the octamer.

Note Added in Proof. Similar experiments with similar results have been conducted in the laboratory of Donald Pious (personal communication).

We thank Elizabeth Albrecht for preparing and analyzing the Hirt supernatants and Jane Radford for preparation of the manuscript. J.P.-Y.T. is a recipient of an American Cancer Society Junior Faculty Research Award. P.A.S. was supported by National Institutes of Health Postdoctoral Training Grant 5 T32 CA09156. P.V.B. was supported by an American Cancer Society postdoctoral fellowship. This work was supported by Grant CA 42771-01 from the National Institutes of Health and Grant RG-1785-A-1 from the National Multiple Sclerosis Society.

1. Rosenthal, A. S. & Shevach, E. (1973) *J. Exp. Med.* **138,** 1194–1212.
2. Houghton, A. N., Thomson, T. M., Gross, D., Bettgen, H. F. & Old, L. J. (1984) *J. Exp. Med.* **160,** 255–269.
3. Kaufman, J. F., Auffray, C., Korman, A. J., Shackelford, D. A. & Strominger, J. (1984) *Cell* **36,** 1–13.
4. Unanue, E. K., Beller, D. I., Lin, C. Y. & Allen, P. L. (1984) *J. Immunol.* **132,** 1–5.
5. Roehm, W. W., Leisbon, A. J., Zlotnik, A., Kappler, J., Marrack, P. & Cambier, J. C. (1984) *J. Exp. Med.* **160,** 679–693.
6. Rosa, F. & Fellous, M. (1984) *Immunol. Today* **5,** 261–262.
7. Gillies, S. D., Folsom, V. & Tonegawa, S. (1984) *Nature (London)* **310,** 594–597.
8. Basta, P. V., Sherman, P. A. & Ting, J. P.-Y. (1987) *J. Immunol.* **138,** 1275–1280.
9. Boss, J. M. & Strominger, J. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9139–9143.
10. Friedman, R. L. & Stark, G. R. (1985) *Nature (London)* **314,** 637–639.
11. Saito, H., Maki, R. A., Clayton, L. K. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5520–5524.
12. Parslow, T. G., Blair, D. L., Murphy, W. J. & Granner, D. K. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2650–2654.
13. Bergman, Y., Rice, D., Grosschedl, R. & Baltimore, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7041–7045.
14. Falkner, F. G. & Zachau, H. G. (1984) *Nature (London)* **310,** 71–74.
15. Queen, C. & Stafford, J. (1984) *J. Mol. Cell Biol.* **4,** 1042–1049.
16. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *J. Mol. Cell Biol.* **2,** 1044–1051.
17. Zagursky, R. J., Baumeister, K., Lomax, N. & Berman, M. C. (1985) *Gene Anal. Tech.* **2,** 89–94.
18. Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. (1983) *Nature (London)* **306,** 557–561.
19. Sompayrac, L. M. & Danna, K. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7575–7578.
20. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
21. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3060.
22. Strauss, F. & Varshavsky, A. (1984) *Cell* **37,** 889–901.
23. Singh, H., Sen, R., Baltimore, D. & Sharp, P. A. (1986) *Nature (London)* **319,** 154–158.
24. Dignam, J. D., Lebowitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
25. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
26. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 122.
27. Staudt, L. M., Singh, H., Sen, R., Wirth, T., Sharp, P. A. & Baltimore, D. (1986) *Nature (London)* **323,** 640–643.
28. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50,** 349–383.
29. Hirt, B. (1967) *J. Mol. Biol.* **26,** 365–369.
30. Landolfi, N. F., Capra, J. D. & Tucker, P. W. (1986) *Nature (London)* **323,** 548–551.
31. Gladstone, P. & Pious, D. (1980) *Somatic Cell Genet.* **6,** 285–298.
32. Accolla, R. S., Carra, G. & Guardiola, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5145–5149.
33. Larhammar, D., Servenius, B., Rask, L. & Peterson, P. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1475–1479.
34. Strominger, J. L. & Bose, J. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5199–5203.
35. Larhammar, D., Hammerling, U., Denaro, M., Lund, T., Flavell, R. A., Rask, L. & Peterson, P. A. (1983) *Cell* **34,** 179–188.
36. O'Sullivan, D. M., Larhammar, D., Wilson, M. C., Peterson, P. A. & Quaranta, V. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4484–4488.
37. Sive, H. L. & Roeder, R. G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6382–6386.
38. Ares, M., Mangin, M. & Weiner, A. M. (1985) *Mol. Cell. Biol.* **5,** 1560–1570.
39. Mattaj, I. W., Leinherd, S., Jiricny, J. & DeRobertis, E. M. (1985) *Nature (London)* **316,** 163–167.
40. Krol, A., Lund, E. & Dahlberg, J. E. (1985) *EMBO J.* **4,** 1529–1535.
41. Cilberto, G., Buckland, R., Cortese, R. & Philipson, L. (1985) *EMBO J.* **4,** 1537–1543.