Functional Roles of the Transcription Factor Oct-2A and the High Mobility Group Protein I/Y in HLA-DRA Gene Expression

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Summary

The class II major histocompatibility complex gene HLA-DRA is expressed in B cells, activated T lymphocytes, and in antigen-presenting cells. In addition, HLA-DRA gene expression is inducible in a variety of cell types by interferon-γ (IFN-γ). Here we show that the lymphoid-specific transcription factor Oct-2A plays a critical role in HLA-DRA gene expression in class II–positive B cell lines, and that the high mobility group protein (HMG) I/Y binds to multiple sites within the DRA promoter, including the Oct-2A binding site. Coexpression of HMG I/Y and Oct-2 in cell lines lacking Oct-2 results in high levels of HLA-DRA gene expression, and in vitro DNA-binding studies reveal that HMG I/Y stimulates Oct-2A binding to the HLA-DRA promoter. Thus, Oct-2A and HMG I/Y may synergize to activate HLA-DRA expression in B cells. By contrast, Oct-2A is not involved in the IFN-γ induction of the HLA-DRA gene in HeLa cells, but antisense HMG I/Y dramatically decreases the level of induction. We conclude that distinct sets of transcription factors are involved in the two modes of HLA-DRA expression, and that HMG I/Y may be important for B cell–specific expression, and is essential for IFN-γ induction.

Class II molecules of the MHC play a central role in the immune response by presenting processed peptides of foreign antigen to T helper cells and by participating in the thymic selection of T lymphocytes (1). The α and β chains of the heterodimeric MHC class II molecules are encoded by genes located within the HLA-DR, -DP, and -DQ loci in human (2), with each set of α and β polypeptides forming a distinct class II isotype. The different class II isotypes are generally expressed coordinately, although it is clear that this is not always the case in vivo (3–5). The expression of MHC class II molecules is ordinarily restricted to cells of the immune system, but a variety of cell types can be induced to express MHC class II molecules by several stimuli (reviewed in 6). Of these, the cytokine IFN-γ is among the most potent and well studied (7, 8). Aberrant expression of MHC class II molecules may be important in the pathogenesis of autoimmune disorders (9, 10) while defective expression of class II molecules is the basis for a subset of severe combined immunodeficiency diseases known as the bare lymphocyte syndrome (11, 12).

Analysis of the DNA sequence requirements for both B cell–specific expression and IFN-γ induction of the HLA genes reveal a complex organization of regulatory elements (reviewed in 6, 13). The proximal 160 bp of MHC class II gene promoters contain conserved DNA elements called the X, Y, and Z/W/H boxes, each of which are required for transcription (14–17). The Y box is an inverted CAAT box and a number of proteins that specifically interact with it have been identified (18, 19).

The X box is found 18–20 bp upstream of the Y box. The sequences separating these elements are variable, but the length of the sequence is both conserved and critical for the transcriptional activity of the genes (17, 20). The X box can be further subdivided into two motifs: the X1 and X2 boxes, which bind distinct types of sequence-specific DNA-binding proteins (21–28).

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A region termed W, which contains multiple potential control elements called Z, H, V, or S, exhibits a degree of sequence conservation when the different class II MHC sequences are aligned (29–34). An additional element, called J, has recently been described, which appears to be important for induction of different class II MHC genes by IFN-γ (35). In addition to these regulatory elements, which are found in all of the class II genes from mice and humans, the human DRA and DQB gene promoters contain a consensus binding site for the transcriptional activators Oct-1 and Oct-2 (14, 36–40).

The proximal promoter is sufficient in directing both B cell–specific and inducible expression of class II genes in transient transfection assays. Most of the DNA-binding proteins that interact with conserved class II MHC regulatory elements in vitro are ubiquitously expressed in vivo and the status of MHC class II transcription correlates with promoter occupancy in vivo. In vivo footprinting of the DRA promoter in uninduced class II–negative HeLa cells shows little protection of conserved motifs, while the regulatory DNA elements in class II–positive B cells are fully protected (40, 41). Induction of class II expression by treatment of HeLa cells in IFN-γ leads to increased protection at the same sites as those protected in class II positive B cells.

The octamer binding site of the HLA-DR promoter has been shown to be required for constitutive expression in B cells but not for IFN-γ induction (16, 42). We have confirmed these findings and we have shown that an octamer-binding site in the HLA-DQB promoter (38), is not required for transcriptional activation. In addition, we have carried out antisense RNA experiments to show that the Oct-2A protein is required for B cell–specific expression of the endogenous HLA-DRA gene.

The high mobility group (HMG)[1] protein HMG I/Y was previously shown to be required for virus induction of the IFN-β gene (43, 44). HMG I/Y facilitates the binding of two distinct transcription factors, NF-κB and ATF-2, and also appears to function as an architectural component of an inducible enhancer complex. Here, we show that HMG I/Y binds to multiple sites within the HLA-DRA promoter, that HMG I/Y and Oct-2A synergize in transfection experiments and that the two proteins cooperatively bind to DNA in vitro. In addition, using antisense RNA experiments we show that HMG I/Y is required for IFN-γ induction of the HLA-DRA promoter. Thus, HMG I/Y may be involved in the assembly of two distinct transcriptional activation complexes on the HLA-DRA promoter, one for B cell–specific expression, and the other for IFN-γ inducible expression.

Materials and Methods

Cell Culture and Flow Cytometry. HeLa cells were grown in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics. Human Raji, Jijoye, and Jurkat cells were grown in RPMI 1640 with 10% fetal bovine serum, 2 mM glutamine, and antibiotics. For induction with IFN-γ HeLa cells were incubated with recombinant IFN-γ (Genentech, South San Francisco, CA) at 500 U/ml for 48 h. The following monoclonal antibodies were used for flow cytometric analysis: LB3.1 (anti-DR), B7/21 (anti-DP), and Genox 3.53 (anti-DQ). These antibodies are all of the IgG1 subclass.

Recombinant DNA and Proteins. The plasmids DRA300CAT and DQB2500CAT have been described (27). A.S. hXBP-1 and a.s.fos. plasmids have also been previously described (28). A.S. oct-1 and A.S. oct-2A were synthesized by subcloning the entire Oct-1 or Oct-2A cDNAs in the reverse orientation behind the CMV promoter in the pcDNA cloning vector. DRA300 mutant promoter–chloramphenicol acetyl transferase (CAT) constructs were made by subcloning the mutated promoter fragments from the Bluescript constructs (Stratagene Inc., La Jolla, CA), described under site-directed mutagenesis below, into the XbaI site of the promoterless plasmid pCATBasic (Promega Corp., Madison, WI). All constructs were confirmed by dideoxy sequencing. *Escherichia coli* expressed HMG I/Y protein was prepared as described (43). Recombinant Oct-2 protein was kindly provided by M. Tanaka (Cold Spring Harbor Laboratory).

Site-directed Mutagenesis. All site-specific mutations in HMG I/Y binding sites were generated by PCR using as template the BSDRA300 plasmid (26). To mutagenize the octamer motif, oligos

44a: (5'AGAGTAATTGATGGGCATTTTAATGG-3')
and 44b: (5'-CCATTAAAATGCCCATCAATTACTCT-3'),

with bold type denoting mutated bases, were used. For mutagenizing the AT-rich D-box region, oligos

45a: (5'ATCTCAAAATATGGGTCTGATTGGCCA-3')
and 45b: (5'-TGGCCAATCAGACCCATATTTTGAGAT-3')

were used. Each of these mutagenizing oligos was used in a first PCR reaction with either the T3 or the T7 Bluescript primers. The two PCR products were purified by Geneclean II (Bio 101, La Jolla, CA), mixed in equimolar amounts and used in a third PCR reaction with the T7 and the T3 primers. The final PCR product was digested with EcoRI and HindIII and subcloned into the EcoRI-HindIII site of the Bluescript KS/+ plasmid. An additional mutant:

(5'-ATCTCCAGATATGGGTCTGATTGGCCA-3')

was generated spuriously. Double mutants were generated by exploiting a unique MscI restriction site between the octamer and the D box to swap fragments between the single mutants. All mutations were confirmed by dideoxy sequencing.

Transfections and CAT Assays. Jijoye and HeLa cells were transfected using lipofectamine (Gibco, Gaithersburg, MD) according to the manufacturer's instructions, while Jurkat cells were transfected using the DEAE dextran transfection method. For assessing the activity of mutant DRA promoters, Jijoye and HeLa cells were transfected with 1 μg of the mutant promoter-CAT constructs. The β galactosidase expression vector, pSV-β-gal (Promega) was included as an internal control to monitor transfection efficiency. Cell extracts were made 48 h after transfection, normalized for β-galactosidase activity, and used in CAT assays as described below. For Jurkat cells, 10^7 recipient cells were washed extensively with serum-free media. 20 μg of cesium chloride–purified, supercoiled plasmid DNA was added to the cells in a 1-ml vol of serum-free RPMI 1640 containing 200 μg of DEAE Dextran (Pharmacia Fine Chemicals, Piscataway, NJ). 5 μg of plasmid pXGH5, a mammalian expression vector encoding human growth hormone, was cotrans-

[1] *Abbreviations used in this paper:* CAT, chloramphenicol acetyl transferase; EMSA, electrophoretic mobility shift assay; HMG, high mobility group.

fected with reporter constructs to control for variability in transfection efficiency. The cells were incubated for 1 h at 37°C before addition of medium. 48 h after transfection, the cells were washed with serum-free media and pelleted. The cells were resuspended in 300 μl of 0.25 M Tris and freeze-thawed three times. After centrifugation in a microfuge to remove debris, 150 μl of the cell extract was incubated with 20 μl 10 mM acetyl coenzyme A (Pharmacia Fine Chemicals) and 2 μl of [^{14}C]chloramphenicol (New England Nuclear, Boston, MA) (49 mCi/mmol, 0.1 mCi/ml) for 4 h at 37°C. The chloramphenicol was then extracted with 1 ml ethyl acetate, speed vacuum dried, and spotted onto TLC plates. After the solvent front was allowed to travel three-quarters of the length of the plate, the plate was removed from the chromatography tank, allowed to dry, and subjected to autoradiography. For quantitation of CAT activity, appropriate slices of the TLC plates were analyzed by liquid scintillation counting.

Gel Retardation Analysis. Jijoye and HeLa whole-cell extracts for gel shift analysis were prepared using a modification of a protocol (45). In brief, 3–5 × 10^6 cells were pelleted, washed in ice-cold PBS, then resuspended in 100 μl of 20 mM Hepes, pH 7.9, 200 mM KCl, 1 mM DTT, 1 mM EDTA, 0.1 mM PMSF, 20% glycerol, and 0.1% NP-40, and incubated with periodic agitation for 1 h at 4°C. The cells and large debris were pelleted by 10-min 14,000-rpm centrifugation in a microfuge at 4°C, and the supernatant, containing proteins solubilized from both the nucleus and the cytosol in the extraction media, removed, aliquoted, quick-frozen in liquid nitrogen, and stored at −80°C. For nuclear extract preparation, 500 ml of log phase cells was pelleted at 2,000 rpm for 10 min. The cell pellet was washed twice in ice-cold PBS and resuspended in 25 ml nuclear isolation buffer I (10 mM Tris-HCl, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, and 1 mM DTT). 0.3 ml of ice-cold 10% NP-40 was added dropwise (while vortexing at lowest setting) and incubated on ice for 20 min. The cell lysate was layered onto 12 ml of ice-cold nuclear isolation buffer I containing 1.7 M sucrose and centrifuged at 13,000 *g* for 15 min in an SW27 rotor (Beckman Instruments, Inc., Palo Alto, CA). Purified nuclei were then resuspended in 3 ml of ice-cold 20 mM Hepes, pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT, and homogenized with 10 strokes of a Dounce homogenizer on ice. The suspension was then rocked for 30 min at 4°C and centrifuged for 30 min at 25,000 *g* in an SS34 rotor (Beckman Instruments). The supernatant was then dialyzed against 150 ml of transcription buffer (-rNTPs): 12 mM Hepes, pH 7.9, 12% glycerol, 0.3 mM DTT, 0.12 mM EDTA, and 60 mM KCl for 5 h. $MgCl_2$ was omitted from those preparations to be used for gel-retardation assay, as $MgCl_2$ has been found to inhibit gel retardation. For gel-shift analysis, ^{32}P end-labeled probes (5,000–15,000 cpm) were incubated in a 10-μl vol with pure recombinant proteins or extracts on ice for 25 min. Binding condition for recombinant HMG I/Y was 10 mM Tris.Cl (pH 7.6), 50 mM NaCl, 5% glycerol, 1 mM EDTA, and 100 ng of poly(dG.dC). Poly(dG-dC) was always used since HMG I/Y binding is sensitive to poly(dI-dC). Octamer protein–binding reactions were in 20 mM Hepes pH 7.9, 2 mM DTT, 8% glycerol, 40 mM KCl, 0.01% NP-40, and 100–500 ng of poly(dG.dC) or poly(dI-dC). The total amounts of protein in reactions containing exogenous HMG I were kept constant by using BSA or recombinant IFN-γ. Antibody to HMG I/Y was generated as described (43) and an anti-βgal antibody was purchased (Promega), 1 μl antibody was used per reaction. Reactions were analyzed by electrophoresis in a nondenaturating 5% polyacrylamide gel in 0.5 × Tris-buffered EDTA. Bands were quantitated by densitometry. The Y + OCT probe has the sequence:

5'TCTCAAAATATTTTTCTGATTGGCCAAAGAGTAATT-GATTTGCATTTTAAT3',

the DRA-OCT oligo:

5'-AGAGTAATTGATTTGCATTTTAATGG-3',

and the DRA-IS oligo:

5'ATCTCAAAATATTTTTCTGATTGGCCA-3'.

DNaseI Footprint Analysis. Both strands of the DRA proximal promoter were subjected to DNaseI footprint analysis using the plasmid pBSDRA300 which contains nucleotides −268 to +29 of the DRA gene inserted into the pBluescript KS vector between the HindIII and EcoRI restriction sites (26). Strand-specific labeling was achieved by labeling after digestion with either HindIII or EcoRI, followed by complete digestion with the other restriction enzyme. The radiolabeled DRA proximal promoter was then purified by elution after gel electrophoresis and the specific activity of the fragment determined by scintillation counting. Footprinting reactions were performed in 50-μl binding reactions containing 10,000 cpm of purified probe with increasing amounts of recombinant HMG I protein for 30 min at room temperature. Binding reactions were carried out in a buffer containing 25 mM Hepes, pH 7.6, 50 mM NaCl, 1 mM DTT, 1 mM EDTA, 10 μg of BSA, 0.01% NP-40, and 1 μg of poly dG/dC as a nonspecific competitor DNA. DNA was fragmented for 1 min in a final concentration of 4 mM $MgCl_2$ using 0.2 U of DNaseI. After 1 min, the reaction was terminated with a solution containing 2% SDS, 200 mM EDTA, and 1 μg of salmon sperm DNA. After phenol-chloroform extraction, reaction mixtures were analyzed by electrophoresis on an 8% polyacrylamide sequencing gel containing 8 M urea. A G + A sequencing ladder was also added for orientation after developing the autoradiograph.

In Vitro Transcription/Translation. In vitro translated Oct-1 protein was made from the pBSOct-1+ plasmid (W. Herr, Cold Spring Harbor Laboratory) using the TNT coupled rabbit reticulocyte lysate system (Promega) according to the manufacturer's instructions.

Results

Differential Requirement of the Octamer-binding Protein Oct-2 for the B Cell–specific Expression of the HLA-DRA and HLA-DQB Genes. Among the human class II MHC genes, only the HLA-DRA and HLA-DQB promoters have been found to contain an octamer-binding site (36, 38; our unpublished observations). In the DRA promoter the octamer element is located between −52 and −45 bp upstream from the start site of transcription, while the octamer element in the DQB promoter is located between −611 and −604 (see Fig. 1 *A*). Previous studies have established that the octamer motif in the HLA-DRA promoter binds both Oct-1 and Oct-2 and mutations that abolish in vitro binding adversely affect expression in B cells (16, 42, 46; and data not shown). Since both Oct-1 and Oct-2 proteins are expressed in B cells, we were interested in determining whether one or both of these proteins are required for HLA-DRA expression. Thus, we cotransfected expression vectors that direct the synthesis of either Oct-1 or Oct-2A antisense RNA with the HLA-DRA reporter construct. While the vector encoding antisense Oct-1 RNA had no effect on either DRA or DQB promoter ac-

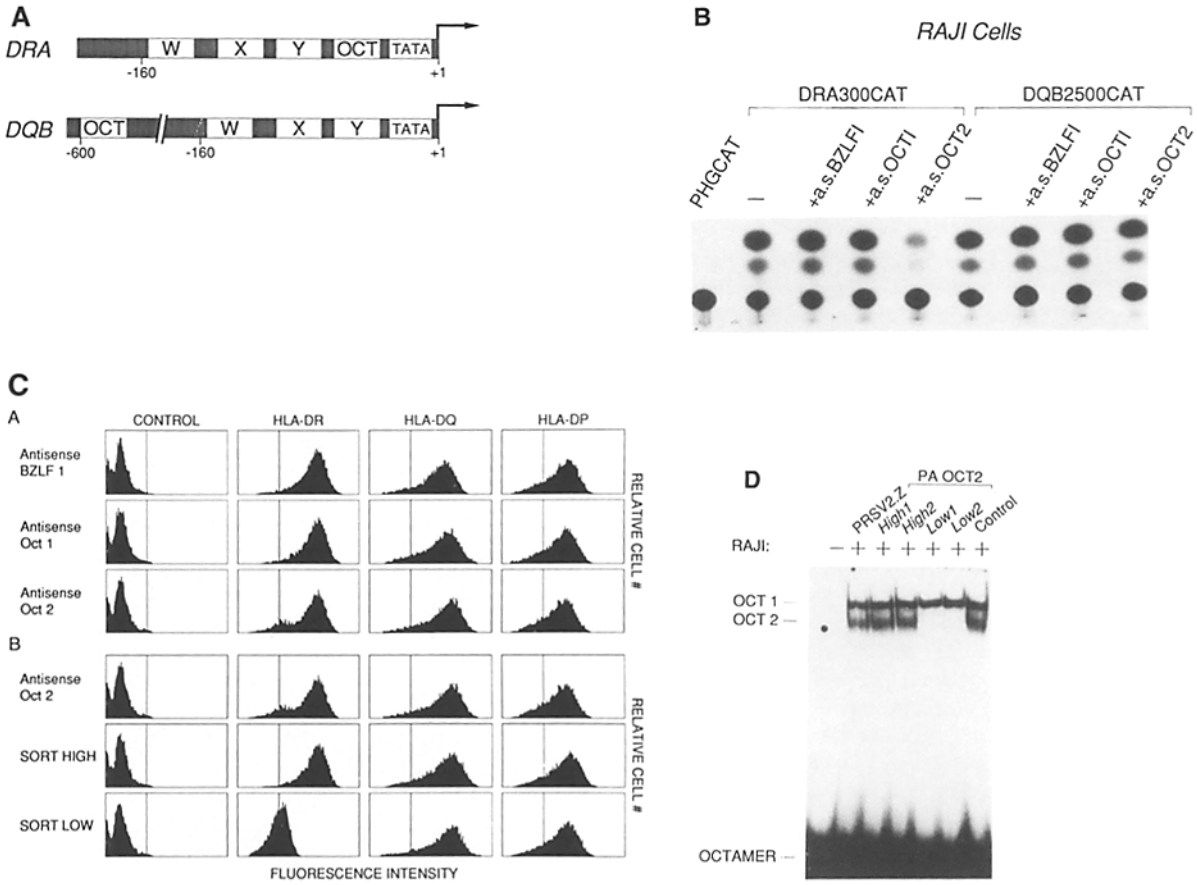

Figure 1. Antisense Oct-2 RNA blocks the expression of the HLA-DRA, but not the -DQB promoter in Raji cells. (*A*) Diagram showing the location of octamer-binding sites within the human class II MHC gene promoters: HLA-DRA (−52 to −45 bp) and HLA-DQB (−611 to −604 bp). (*B*) Effect of expression of antisense Oct-2 RNA on HLA-DRA and DQB promoter activity as assessed by CAT assay. Raji cells were transfected with the indicated reporter constructs (pHGCAT is a promoterless CAT plasmid). Lanes marked - were from cells transfected with only the reporter construct. In the remaining lanes the cells also received the indicated antisense vector. a.s. BZLF1 directs the expression of an antisense BZLF1 RNA (the immediate early response transcription factor Zebra, involved in the latent/lytic transition of EBV; a.s. Oct1 directs the expression of antisense Oct1 RNA; a.s. Oct2 directs the expression of antisense Oct2A RNA. (*C*) The effect of antisense Oct1 or Oct2 RNA on cell-surface expression of endogenously encoded MHC class II molecules. Raji cells were transfected with the vectors described in *B* and cell-surface expression of the HLA-DR, -DQ, and -DP molecules was assessed by flow-cytometric analysis using isotype-specific monoclonal antibodies (*subpanel A*). Synthesis of antisense Oct2 RNA resulted in the specific down-regulation of HLA-DR expression. The subpopulation of cells expressing reduced levels of HLA-DR was purified by cell-sorting (*subpanel B*). The same cells that expressed reduced HLA-DR, expressed wild-type levels of HLA-DQ and -DP. (*D*) Raji cells were transfected with the control antisense vector pRSV2.Z or the antisense Oct2 expression vector. Cells transfected with the antisense Oct2 expression vector were then sorted into HLA-DR high and low populations by flow cytometry, based upon cell-surface HLA-DR expression levels. Whole cell extracts were prepared from these cells and assessed for the presence of octamer-binding proteins by EMSA. The lane marked *Control* contains extract from unsorted, p.a. Oct2-transfected Raji cells. Cells expressing decreased levels of cell-surface HLA-DR (lanes marked *Low*) show no detectable levels of Oct2.

tivity (in the B lymphoblastoid cell line Raji), the vector encoding antisense Oct-2A RNA strongly down-regulated expression of the DRA (but not the DQB) promoter in these cells (Fig. 1 *B*).

To further assess the significance of the octamer-binding proteins on the transcription of the class II MHC genes, we tested the effect of antisense Oct-1 and Oct-2A RNA production on the cell-surface expression of the endogenous class II molecules in the B cell line Raji (Fig. 1 *C*). Raji cells were transiently transfected with either the antisense Oct-1 or Oct-2A expression vector and then analyzed for surface class II antigen expression by flow cytometric analysis using isotype-specific monoclonal antibodies. It is important to note that cell-surface expression of any of the class II isotypes requires transcription and translation of both the α and β chain genes. Therefore, a reduction in expression of either an α or β chain gene at a particular locus will result in decreased cell-surface expression of that particular isotype. Significantly, Raji cells transfected with the antisense Oct-2A expression vector exhibit a specific down-modulation of HLA-DR antigen expression in a subpopulation of cells (Fig. 1 *C*, *subpanel A*). In contrast, vectors encoding antisense Oct-1 and the control antisense Zebra RNA had no effect on cell-surface expression of any of the class II isotypes.

To further characterize this phenomenon, the subpopulation of Raji cells transfected with the antisense Oct-2A expression vector (designated: HLA-DRA sort low) were purified by cell sorting and shown to express wild-type levels

of HLA-DQ and -DP (Fig. 1 *C*, *subpanel B*). Whole-cell extracts were then prepared from the sorted populations of cells transfected with antisense Oct-2A expression vector and analyzed by gel mobility shift assay for expression of the octamer-binding proteins (Fig. 1 *D*). The sorted subpopulation of Raji cells expressing markedly decreased levels of cell surface HLA-DR molecules showed reduced levels of Oct-2 protein (lanes marked *Low*), while the subpopulation of antisense Oct-2A expression vector transfected cells that express high levels of surface HLA-DR retain normal levels of Oct-2 protein (lanes marked *High*). Thus, there is a correlation between reduced cell-surface expression of HLA-DR molecules and reduced amounts of Oct-2 protein. The subpopulation of cells transfected with antisense Oct-2A expression vector that express wild-type levels of HLA-DR presumably did not incorporate the expression vector.

The Octamer-binding Protein Oct-2A Is Not Required for IFN-γ Induction of the HLA-DRA Gene. To further investigate the role of the octamer-binding site in IFN-γ induction of the HLA-DR promoter, we examined the effects of mutations in this site on the expression of the DRA300CAT reporter in B cells and in HeLa cells. We substituted the TT bases for GG in the octamer element as shown in Fig. 2 *A*. This mutation drastically reduced the expression of the reporter

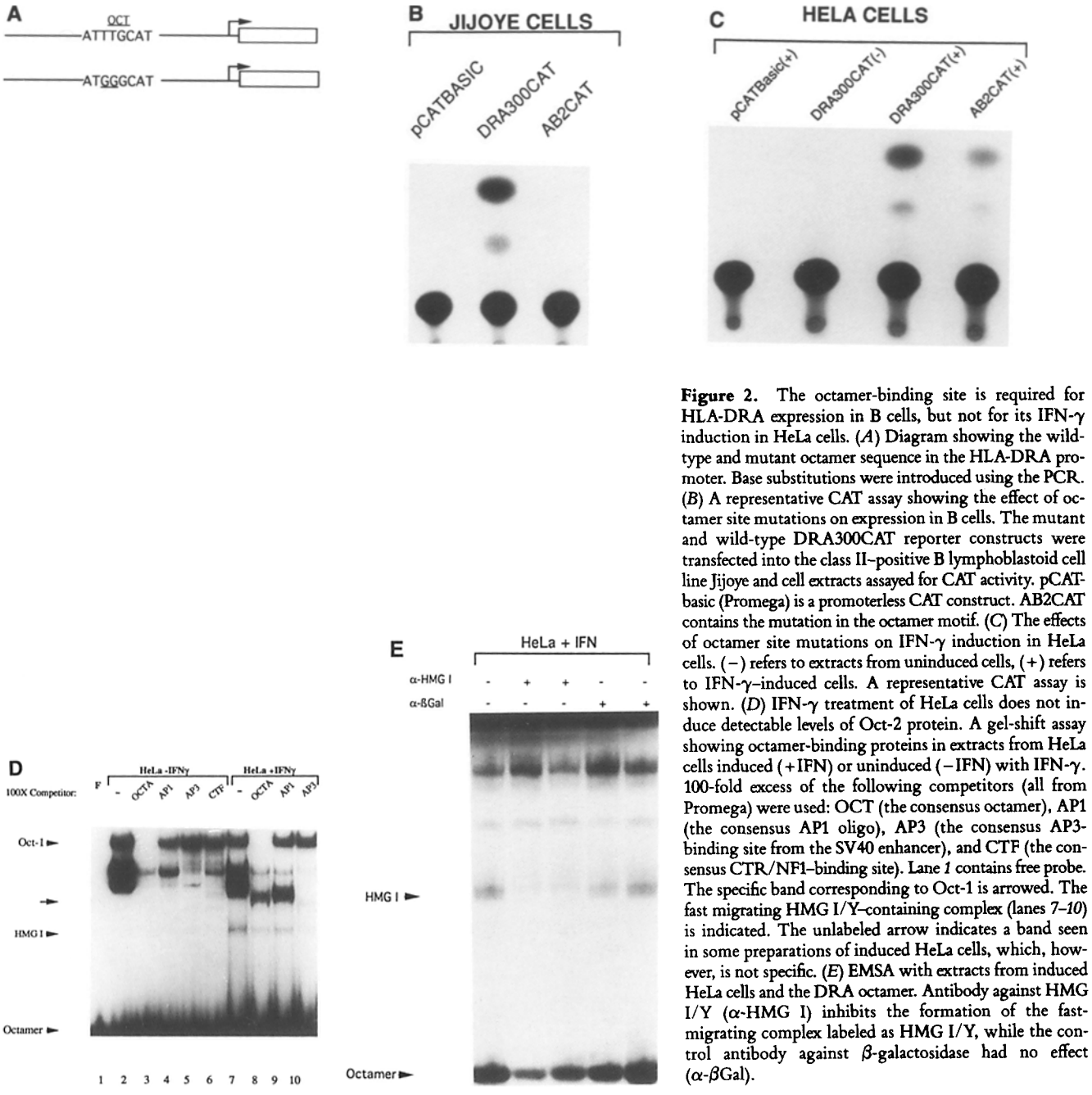

Figure 2. The octamer-binding site is required for HLA-DRA expression in B cells, but not for its IFN-γ induction in HeLa cells. (*A*) Diagram showing the wild-type and mutant octamer sequence in the HLA-DRA promoter. Base substitutions were introduced using the PCR. (*B*) A representative CAT assay showing the effect of octamer site mutations on expression in B cells. The mutant and wild-type DRA300CAT reporter constructs were transfected into the class II–positive B lymphoblastoid cell line Jijoye and cell extracts assayed for CAT activity. pCAT-basic (Promega) is a promoterless CAT construct. AB2CAT contains the mutation in the octamer motif. (*C*) The effects of octamer site mutations on IFN-γ induction in HeLa cells. (−) refers to extracts from uninduced cells, (+) refers to IFN-γ–induced cells. A representative CAT assay is shown. (*D*) IFN-γ treatment of HeLa cells does not induce detectable levels of Oct-2 protein. A gel-shift assay showing octamer-binding proteins in extracts from HeLa cells induced (+IFN) or uninduced (−IFN) with IFN-γ. 100-fold excess of the following competitors (all from Promega) were used: OCT (the consensus octamer), AP1 (the consensus AP1 oligo), AP3 (the consensus AP3-binding site from the SV40 enhancer), and CTF (the consensus CTR/NF1–binding site). Lane *1* contains free probe. The specific band corresponding to Oct-1 is arrowed. The fast migrating HMG I/Y–containing complex (lanes *7–10*) is indicated. The unlabeled arrow indicates a band seen in some preparations of induced HeLa cells, which, however, is not specific. (*E*) EMSA with extracts from induced HeLa cells and the DRA octamer. Antibody against HMG I/Y (α-HMG I) inhibits the formation of the fast-migrating complex labeled as HMG I/Y, while the control antibody against β-galactosidase had no effect (α-βGal).

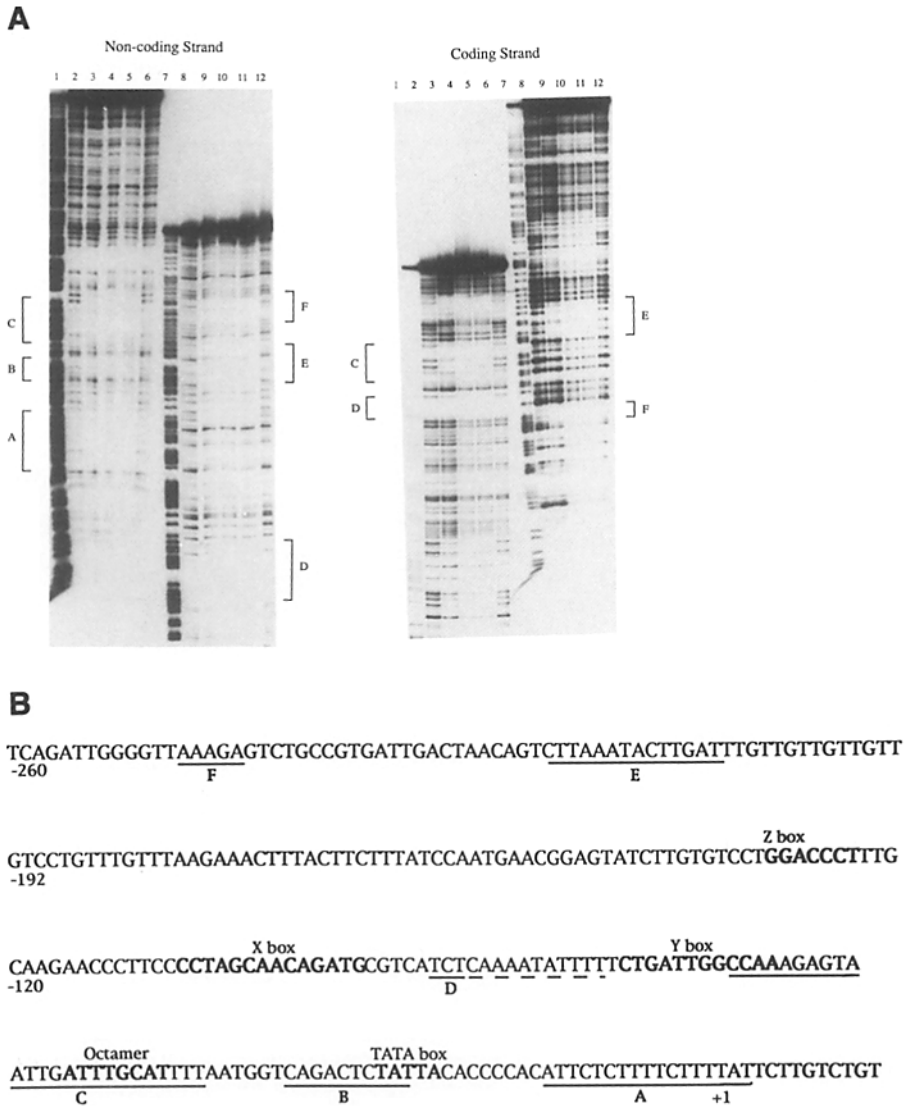

Figure 3. Identification of HMG I/Y-binding sites in the HLA-DRA promoter. (*A*) In vitro DNase I footprint analysis of regions of the HLA-DRA proximal promoter that are protected by binding of recombinant HMG I/Y. The noncoding strand is end-labeled in subpanel *A* and the coding strand in subpanel *B*. Lanes *1* and *7* are Maxam-Gilbert G + A sequencing ladders. Lanes *2*, *6*, *8*, and *12* are DNaseI-generated fragments in the absence of HMG I/Y. Lanes *3–5* and *9–11* contain increasing amounts of HMG I/Y. Six protected regions are labeled *A–F*. (*B*) Sequence of the HLA-DRA proximal promoter showing the regions footprinted by HMG I/Y binding. Site A coincides with the transcription initiation site, site B with the TATA element, site C with the octamer motif, site D with the A/T-rich interspace region between the X and Y boxes, and two upstream binding sites (E and F) that are located in a region which is dispensable for tissue-specific and inducible expression of the HLA-DRA promoter.

gene in the B cell line Jijoye (Fig. 2 *B*). On the other hand, the same mutations in the octamer-binding site had only a slight effect on the induction of the HLA-DRA promoter by IFN-γ in HeLa cells (Fig. 2 *C*). While the octamer mutation almost completely abolished HLA-DRA promoter-driven CAT activity in B cells, it reduced CAT activity in HeLa cells to between 70 and 80% of wild type (Fig. 2, *B* and *C*; see also Fig. 4 *C*). These observations are in general agreement with earlier reports that DRA reporter constructs containing mutations in the octamer element have impaired activities in B cells but not in HeLa cells induced with IFN-γ (16, 42).

HeLa cells do not normally express Oct-2 protein (46–48). We, however, wanted to investigate the possibility that Oct-2 plays a role in IFN-γ–mediated induction of HLA-DRA gene expression in HeLa cells. We therefore assayed for de novo Oct-2 protein synthesis in IFN-γ–treated HeLa cells by gel-shift analysis using the DRA-octamer as a probe. As shown in Fig. 2 *D*, we found no evidence of de novo Oct-2 synthesis in HeLa cells induced with IFN-γ. This observation strongly supports the notion that induction of the DRA promoter in HeLa cells occurs via an Oct-2–independent pathway. Thus the modest effects of the octamer mutation seen on inducibility in HeLa cells is more likely to be due to the impairment of another factor, which we feel is likely to be HMG I/Y itself. In support of this model, we have observed in electrophoretic mobility shift assays (EMSA)s that a high mobility complex which forms on the DRA octamer element is enhanced when extracts from IFN-γ–induced HeLa cells are used (Fig. 2 *D*; compare lane *2* with lane 7). This complex appears to contain HMG I/Y or an immunologically related protein as judged by its sensitivity to antisera raised against recombinant HMG I (Fig. 2 *E*). This observation suggests that induction of HeLa cells with IFN-γ enhances the binding of HMG I or an HMG I–containing complex to the DRA octamer and suggests that the slight impairment in IFN-γ inducibility of the AB2CAT reporter may result from inhibition of HMG I/Y binding.

The High Mobility Group Protein HMG I/Y Binds to Multiple Sites in the HLA-DRA Promoter, Including the Octamer-binding Site. Previous studies have in fact shown that the

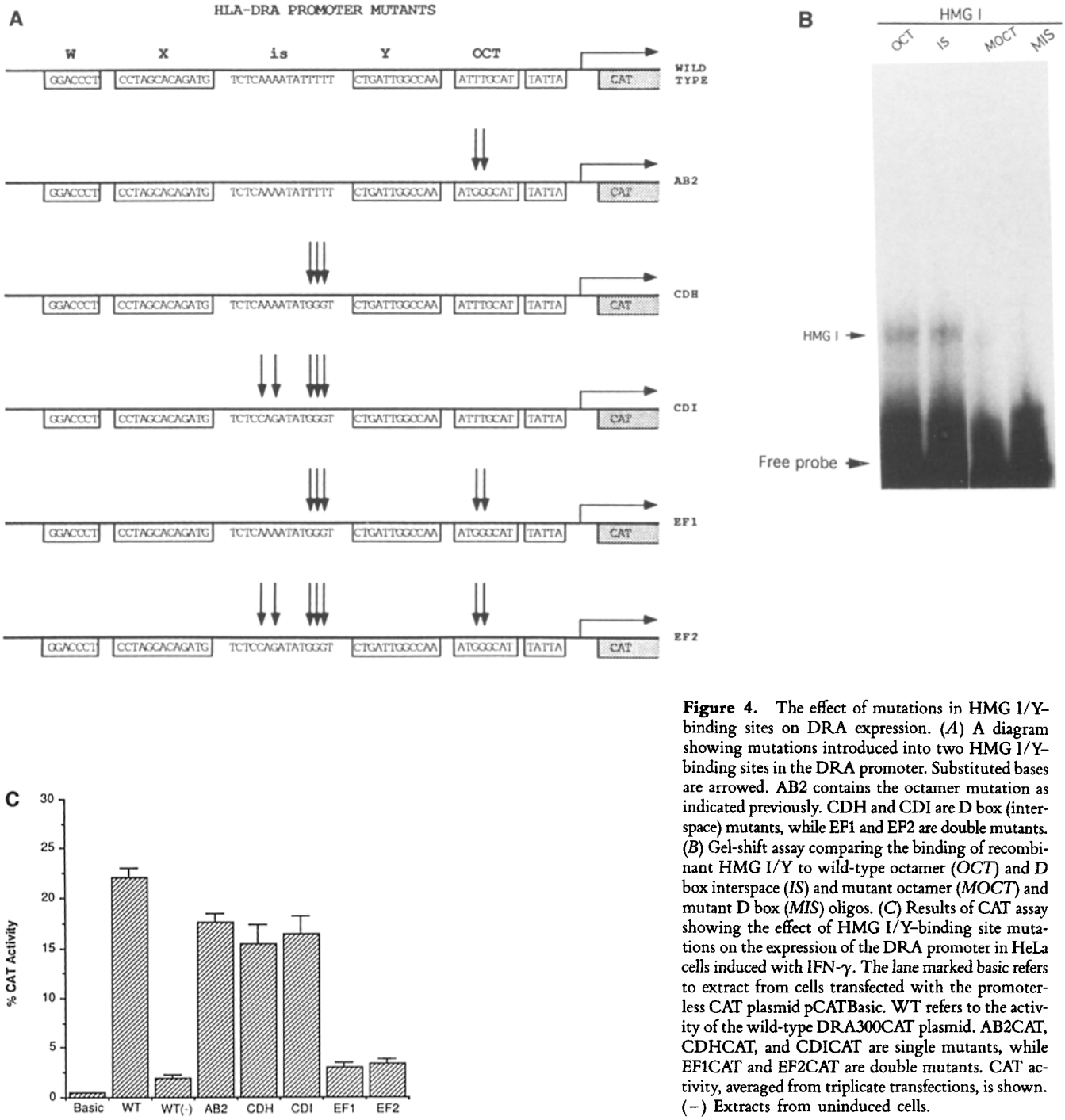

Figure 4. The effect of mutations in HMG I/Y-binding sites on DRA expression. (*A*) A diagram showing mutations introduced into two HMG I/Y-binding sites in the DRA promoter. Substituted bases are arrowed. AB2 contains the octamer mutation as indicated previously. CDH and CDI are D box (interspace) mutants, while EF1 and EF2 are double mutants. (*B*) Gel-shift assay comparing the binding of recombinant HMG I/Y to wild-type octamer (*OCT*) and D box interspace (*IS*) and mutant octamer (*MOCT*) and mutant D box (*MIS*) oligos. (*C*) Results of CAT assay showing the effect of HMG I/Y-binding site mutations on the expression of the DRA promoter in HeLa cells induced with IFN-γ. The lane marked basic refers to extract from cells transfected with the promoterless CAT plasmid pCATBasic. WT refers to the activity of the wild-type DRA300CAT plasmid. AB2CAT, CDHCAT, and CDICAT are single mutants, while EF1CAT and EF2CAT are double mutants. CAT activity, averaged from triplicate transfections, is shown. (−) Extracts from uninduced cells.

HMG I/Y protein can interact with the octamer element of the human Ig L promoter (49). The DRA promoter contains an octamer element, as well as several AT-rich regions, which are potential HMG I/Y-binding sites. To precisely map the HMG I/Y-binding sites within this region we carried out DNAse I footprinting experiments using recombinant HMG I/Y (Fig. 3 *A*). Six binding sites, designated A–F (Fig. 3 *B*), were identified on both the coding and noncoding strands. These sites coincide with: (*A*) the transcription initiation site, (*B*) the TATA-element, (*C*) the octamer motif, (*D*) the A/T-rich region located between the X and Y boxes, and (*E* and *F*) two additional sites between −250 and −200. As would be expected from the known sequence preference of HMG I/Y, all of these binding sites are A/T rich.

The two upstream sites, E and F, do not appear to be essential for HLA-DRA gene expression, since reporter constructs lacking these sites exhibit proper tissue-specific and inducible expression patterns (14, 31, 50). To further charac-

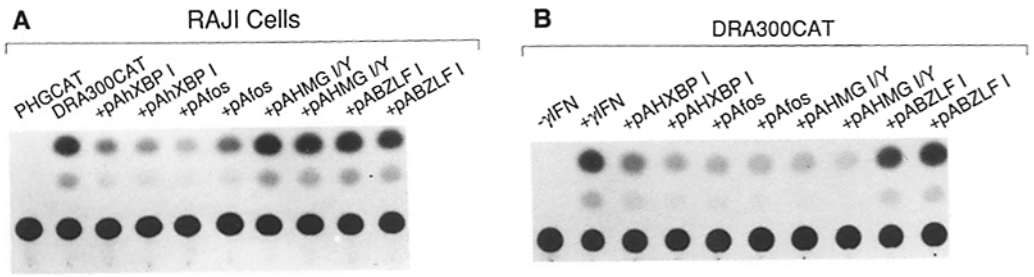

Figure 5. Antisense HMG I/Y RNA inhibits IFN-γ induction of the HLA-DRA gene. (*A*) Raji cells were transiently transfected with the indicated plasmids and CAT expression assessed 48 h after transfection. The lanes marked pHGCAT and DRA300CAT were from cells transfected with those plasmids alone. The other lanes were from cells transfected with both the DRA300CAT reporter plasmid and the indicated antisense expression vector. The plasmids: pAhXBP1, pAfos, and pAHMGI/Y, each contain either the entire or portions of the coding regions of the hXBP1, c-*fos*, and HMG I/Y genes inserted in the antisense orientation in mammalian expression vectors. Antisense hXBP1 and c-*fos* RNA inhibit transcription from the DRA300CAT reporter construct as previously reported (28). Antisense HMG I/Y and BZLF1 RNA have no effect on DRA transcription. Results from duplicate transfections are shown. (*B*) HeLa cells transfected with the DRA300CAT plasmid were induced with recombinant IFN-γ (except the lane marked -IFN-γ). As in *A*, cells were cotransfected with various antisense vectors (as indicated). In these cells, antisense HMG I/Y RNA (in addition to antisense hXBP1 and c-fos RNA) is able to inhibit induction of the gene.

terize these binding sites, we decided to study the effects of mutations that affect HMG I/Y binding to these sites on DRA expression. Unfortunately, the effect of mutations in the A and B boxes could not be tested, since these mutations would be expected to disrupt transcriptional initiation and TATA box function, respectively. The effect of mutations in the octamer (C box) and the D box, were thus studied. We used DRA reporter constructs that contained mutations at these single sites as well as double mutants (Fig. 4 *A*). These mutations abolish HMG I/Y binding in vitro, as shown in Fig. 4 *B*.

The effects of these mutations on B cells, as well as inducible expression in HeLa cells, were studied. As expected, all the constructs containing octamer mutations had severely impaired expression in B cells (Fig. 2 *B*, and data not shown), while the D box mutations had only modest effects on B cell–specific or inducible expression of the HLA-DRA reporter (Fig. 4 *C*, and data not shown). Significantly, however, while neither the octamer mutation nor the D box mutation alone greatly impaired IFN-γ–inducible expression in HeLa cells, constructs containing mutations at both sites showed a severely reduced level of expression (Fig. 4 *C*). These results indicate that multiple HMG I/Y–binding sites may be required for IFN-γ–inducible expression of the DRA promoter.

The Effect of Antisense HMG I/Y RNA on HLA-DRA B Cell–specific and Inducible Expression. We carried out experiments using antisense HMG I/Y RNA to further investigate a possible role of HMG I/Y in constitutive and inducible expression of the HLA-DRA gene. Raji cells were cotransfected with the HLA–DRA300 reporter construct and a plasmid directing the expression of antisense HMG I/Y RNA, and no effect on HLA-DRA expression was observed (Fig. 5 *A*). By contrast, as we previously reported, antisense hXBP-1 and c-*fos* RNA expression decreased transcription from the DRA300CAT reporter construct in these cells (27, 28). These results might suggest that HMG I/Y is not required for B cell–specific expression of HLA-DRA. Alternatively, the antisense RNA might not effectively block the expression of HMG I/Y in B cells.

A dramatically different result was obtained when the an-

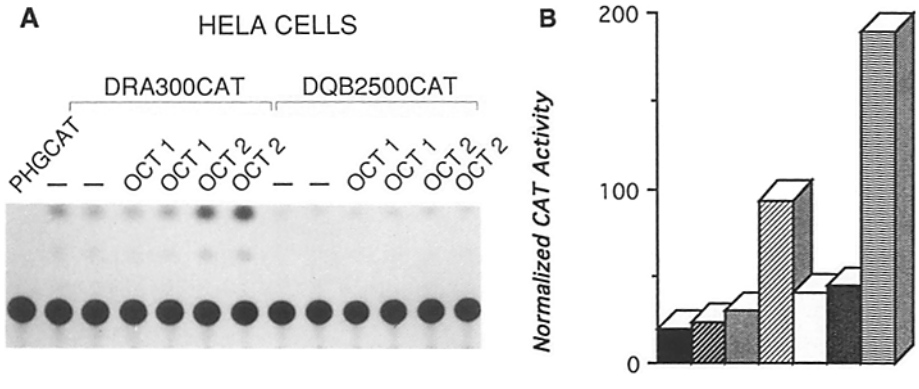

Figure 6. Transactivation of the HLA-DRA promoter by the octamer-binding transcription factor Oct-2A and its cooperation with HMG I/Y. (*A*) Either the DRA300CAT or DQB2500CAT reporter plasmid was transfected into the MHC class II–negative, Oct-2–negative cell line HeLa, alone (lanes marked -) or with mammalian expression vectors encoding Oct1 or Oct2. Overexpression of Oct2 selectively transactivates the HLA-DRA promoter. Results of duplicate transfections are shown. (*B*) Oct-2 transactivates DRA300CAT in the Oct-2–negative, MHC class II–negative cell line Jurkat, and this transactivation is facilitated by HMG I/Y overexpression. Jurkat cells were transfected with DRA300CAT and the indicated expression vectors and CAT activity was assessed 48 h after transfection. The y-axis indicates CAT activity in extracts from these cells relative to CAT activity in DRA300CAT-transfected Jijoye cells (DRA300CAT-mediated activity in Jiyoye cells results in 23% transacetylation). The data are from three independent transfections. ■, pHGCAT; ▨, DRACAT; ▩, DRACAT + Oct-1; ▨, DRCAT + Oct-2A; □, DRACAT + HMG 1; ■, DRACAT + HMG 1 + Oct-1; ▤, DRACAT + HMG 1 + Oct-2A.

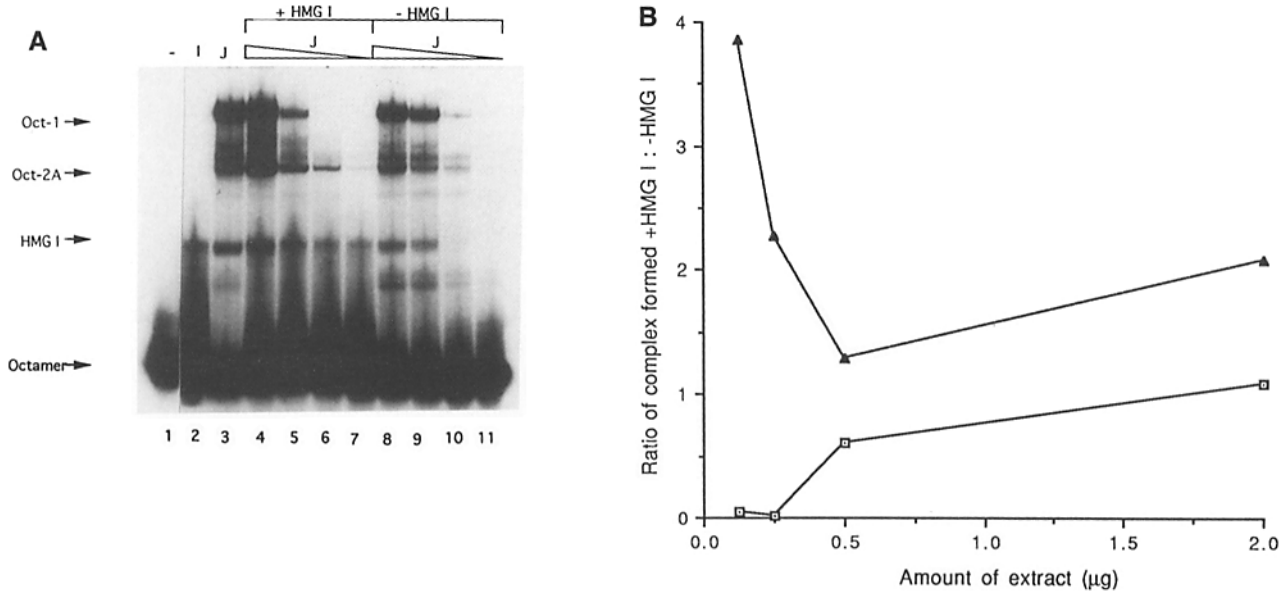

Figure 7. HMG I/Y selectively facilitates interaction of Oct-2 with the HLA-DRA octamer motif in EMSAs. (*A*) The influence of recombinant HMG I on Oct-1 and Oct-2 binding to the HLA-DRA octamer motif was assessed in EMSAs using Jijoye extracts that contain both Oct-1 and Oct-2. Decreasing amounts of Jijoye whole-cell extract (10 μg–0.1 μg) were incubated with a fixed amount of the radiolabeled octamer probe in the presence (lanes *4–7*) or absence (lanes *8–11*) of 0.1 μg of recombinant HMG I. Lane *1* contains free probe, lane *2* contains only recombinant HMG I, lane *3* contains only Jijoye extract (10 μg). The positions of major retarded complexes are indicated by arrows. The recombinant HMG I complex comigrates with a complex observed in Jijoye extracts (which may be endogenous HMG I/Y). The formation of the Oct-2A–containing complex is selectively enhanced in the presence of HMG I, especially at lower concentrations of extract (compare lanes *6* and *7* to lanes *10* and *11*). (*B*) The efficiency of Oct-1 and Oct-2A complex formation in the various conditions were determined by densitometry and the results are shown in this graph. The y-axis represents the ratio of octamer-binding complex formation in the presence of HMG I to that in the absence of HMG I. The x-axis represents the amount of whole-cell extract used in each reaction. —⊡—, OCT 1; —▲—, OCT 2A.

tisense RNA experiments were carried out in HeLa cells induced with IFN-γ. As shown in Fig. 5 *B*, expression of antisense HMG I/Y RNA in HeLa cells abolishes IFN-γ induction of the HLA–DR reporter construct. This observation, in conjunction with the results of the mutations in the HMG I/Y–binding sites of the HLA-DR gene, indicates that HMG I/Y is essential for the induction of HLA-DRA gene expression by IFN-γ.

Oct-2A Is a Transactivator of the HLA-DRA Gene and Cooperates with HMG I/Y. As we have shown above, the Oct-2 protein is required for constitutive expression of the DRA gene in B cells (Fig. 1). To determine whether transfected Oct-2 expression vector can mediate activation of HLA-DRA gene expression in an Oct-2–negative, class II–negative cell line, we cotransfected the HLA–DRA300 reporter construct and an Oct-2A expression vector into either HeLa or Jurkat cells. As shown in Fig. 6 *A*, when increasing amounts of the Oct-2 expression vector were cotransfected with the reporter into HeLa cells, significant levels of CAT activity were observed. The reporter gene was not activated when an Oct-1 expression plasmid was cotransfected with the reporter gene. As expected, the DQB promoter, which we have shown does not require Oct-2 for its expression in B-cells (Fig. 1), is not transactivated by overexpression of either Oct-1 or Oct-2A (Fig. 6 *A*).

Expression of Oct-2A, but not Oct-1, also activated expression of the HLA-DRA reporter plasmid in the class II–negative cell line Jurkat (Fig. 6 *B*). Since HMG I/Y also interacts with the octamer element, we tested the effect of overexpressing this protein on DRA promoter activity in Jurkat cells. We found that overexpression of HMG I/Y alone did not increase the level of HLA-DRA promoter activity. However, coexpression of both HMG I/Y and Oct-2A resulted in a significant increase in the level of HLA-DRA promoter activity compared with the effect of overexpressing Oct-2A alone (Fig. 6 *B*).

HMG I/Y Selectively Facilitates Interaction of Oct-2A with the HLA-DR Octamer Motif. To explore the possible mechanisms by which HMG I/Y facilitates DRA transactivation by Oct-2, we carried out EMSAs to assess the effect of HMG I/Y on the binding of Oct-2 to the octamer element. Initially, we examined the effect of recombinant HMG I/Y on the assembly of nucleoprotein complexes at the octamer site from whole cell extracts. As shown in Fig. 7 *A*, in the presence of low protein concentrations of Jijoye extracts, the binding of the octamer factors Oct-1 and Oct-2 is undetectable (lane *11*). However, addition of a fixed amount of HMG I/Y (0.1 μg) resulted in a specific enhancement of the binding of Oct-2A to the octamer element (lanes *6* and *7*). The binding of the octamer binding proteins Oct-1 and Oct-2B (the alternatively spliced form of Oct-2 which migrates with an intermediate mobility between Oct-1 and Oct-2A; 51, 52) are not facilitated in the presence of additional recombinant HMG I/Y. Indeed, the affinity of Oct-1 is reduced in the presence of HMG I/Y (compare lanes *6* and *10*). Thus the presence of HMG I/Y not only facilitates the binding of Oct-

2A to the octamer element but actually inhibits the binding of other competing proteins such as Oct-1.

To quantitate these effects, we analyzed the abundance of each complex in a representative experiment by densitometric analysis and the results presented in Fig. 7 *B*. These data indicate that the formation of the Oct-2A–containing complex is enhanced approximately fourfold at low concentrations of Jijoye extract. A more striking observation is that the ratio of Oct2A to Oct-1 complex formation is shifted 90- to 100-fold in favor of Oct-2A in the presence of recombinant HMG I/Y. These effects of HMG I/Y may have been due either to the direct influence of HMG I/Y itself, or might have been mediated by other factor(s) present in Jijoye extracts. To distinguish between these possibilities, we performed these experiments using recombinant Oct-1 or Oct-2 proteins. We first tested the influence of HMG I/Y on the binding of recombinant Oct-2A. As shown in Fig. 8 *A*, HMG I significantly enhances the binding of Oct-2A to the HLA-DRA octamer at low Oct-2A concentrations. In sharp contrast, we found that the binding of the in vitro translated Oct-1 protein was inhibited by HMG I/Y, as we have observed with B cell–derived Oct-1 in EMSAs using crude nuclear extracts (Fig. 8 *B*; compare lanes *2–4* with lanes *5–7*). Taken together, these data strongly suggest that HMG I/Y exerts direct and opposing effects on the interaction of Oct-1 and Oct-2 proteins with the octamer element.

Discussion

The MHC class II gene DRA is the most highly expressed of the human class II genes, and the HLA-DR molecule is the predominant restriction element for helper T cell clones (1, 50). Aberrant or unusually high levels of expression of the class II molecules have also been implicated in the pathogenesis of various autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and insulin-dependent diabetes mellitus. The lack of expression of these molecules is the cause of the severe combined immunodeficiency disorder, bare lymphocyte syndrome. The transcriptional regulation of these genes has therefore been the focus of intense investigation (6). These studies of the HLA-DRA promoter have led to the identification of conserved elements within the promoter and a number of DNA-binding proteins which appear to participate in the regulation of the gene (reviewed in 13).

An octamer element has been found in two human class II MHC genes: HLA-DRA and -DQB. While there have been reports that the octamer is important for the constitutive expression of DRA in B cells (31, 42), its role in DQB expression had not been assessed. In this report, we have confirmed the role of the octamer in constitutive expression of the DRA promoter, and have shown that the octamer-binding protein Oct-2A is critical for the expression of the endogenous DRA gene. Neither Oct-1 nor Oct-2 appears to be involved in DQB transcription as assessed by our experiments. It is possible that the positioning of the element in DQB (at about −600 upstream of the transcription start site), compared with its location in the DRA promoter (where it is found just upstream of the TATA box), accounts for this observation. We cannot of course exclude the possibility that the element plays some role in DQB expression at some point during development or in a specific physiologic setting.

We have also investigated the role of another octamer-binding protein, the high mobility group protein HMG I/Y, in HLA-DRA transcription. We have found that HMG I/Y binds to six sites between −300 bp and the transcription start site of the HLA-DRA promoter. These include: two sites upstream of −200 bp, an AT-rich palindromic motif situated between the X and the Y boxes (which we term the D box), the octamer motif, the TATA box, and a site that overlaps the transcription initiation site (which we call the A box). It is of interest that some of these sites coincide with well-defined *cis* elements that bind either well-defined regula-

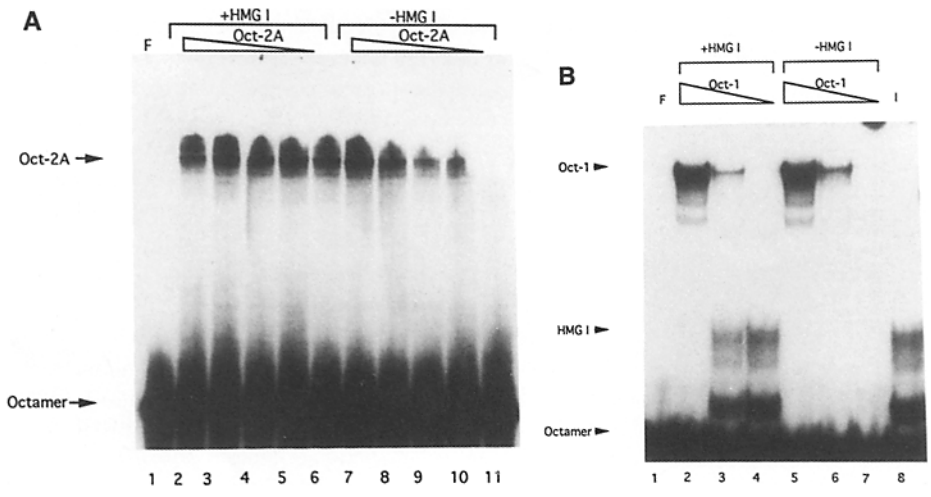

Figure 8. Influence of HMG I/Y on the binding of Oct-2 and Oct-1. (*A*) The influence of recombinant HMG I on recombinant Oct-2/HLA-DRA octamer complex formation was assessed by EMSA. Decreasing amounts (10 ng–0.5 ng) of recombinant Oct-2A protein were incubated with radiolabeled DRA octamer in the presence (lanes *2–6*) or absence (lanes *7–11*) of 10 ng HMG I. Total amounts of protein were kept constant. Lane *1* contains free probe. The formation of the Oct-2A–containing complex is selectively enhanced in the presence of HMG I, especially at lower concentrations (compare lanes *5* and *6* to lanes *10* and *11*). (*B*) EMSA showing decreasing amounts of in vitro translated Oct-1 protein incubated with (lanes *2–4*) or without (lanes *5–7*) 100 ng of recombinant HMG I. Lane *8* contains recombinant HMG I alone. Lane marked *I* contains recombinant HMG I alone and lane marked *F* contains free probe.

tory DNA-binding proteins or components of the general transcriptional machinery.

In a series of experiments using mammalian expression vectors directing the expression of sense or antisense HMG I/Y RNA, and using site-directed mutants of the promoter to assess the importance of individual HMG I/Y-binding sites, we show here that HMG I/Y is essential for IFN-γ induction of the DRA gene in HeLa cells. Interestingly, treatment of HeLa cells with IFN-γ appears to enhance HMG I/Y-binding activity in EMSAs, strengthening the notion that HMG I/Y plays a role in mediating IFN-γ–inducible expression of the DRA gene. We do not at this point know why IFN-γ induction enhances the HMG I/Y-containing complex, although it does not appear to result from induction of HMG I/Y itself (our unpublished observations).

In contrast, expression of antisense HMG I/Y RNA did not affect DRA promoter activity in transiently transfected B cell lines which constitutively express class II genes. Although this might be interpreted to indicate that HMG I/Y is not involved in DRA transcription in B cells, we feel that this is not likely in view of the strong cooperativity between Oct-2A and HMG I/Y that we have observed both in vitro and in vivo in these experiments. Rather, we suspect that the inability of antisense HMG I/Y RNA to block DRA expression in B cells may be due to the presence of higher levels of HMG I/Y in these cell lines compared with HeLa cells.

We are also intrigued by the finding that DRA reporter constructs containing simultaneous mutations in two of the HMG I/Y–binding sites (the octamer and the D box) exhibit severely impaired IFN-γ inducibility, while promoters containing single mutations in these sites show near-normal levels of activity. The observation that mutations in multiple HMG I/Y–binding sites are needed to impair IFN-γ–induced expression suggests that the multiple HMG I/Y molecules bound at various sites within the DRA promoter may to an extent be redundant, providing the generic function of facilitating assembly of the active transcription complex. This would almost certainly be achieved by interactions between HMG I/Y molecules bound at distinct sites or between HMG I/Y molecules and critical activator proteins (such as Oct-2A in B cells).

The observation that both HMG I/Y and Oct-2 interact with the DRA octamer prompted us to investigate possible interactions between these proteins. Cotransfection of an HMG I/Y expression vector with either Oct-1 or Oct-2A expression vectors into the class II–negative cell line Jurkat indicated that HMG I/Y can selectively facilitate the ability of Oct-2A to transactivate the DRA promoter. Furthermore, HMG I/Y selectively facilitates the binding of Oct-2A, but not Oct-1, to the octamer element in EMSAs.

A ternary complex of Oct-protein/HMG I/octamer was not apparent in our gel shift assays. The presence of HMG I/Y did not significantly alter the migration of the Oct-2 or Oct-1 complexes, and the inclusion of antibody to HMG I/Y in the reactions did not result in a significant change in mobility of the complex (Figs. 7 *A*, 8 *A*, and 8 *B*, and data not shown). This observation may be due to the small size of HMG I/Y or may be due to instability of the HMG I/Y containing complex under the EMSA conditions. There is indeed precedence for proteins that enhance the binding of sequence-specific transcription factors to DNA without being retained in the complex during nondenaturing electrophoresis. For instance, Phox 1 enhanced the binding of serum response factor to DNA without altering the mobility of SRF–DNA complexes (53). Like the DNA-bending protein, HMG-1 facilitated the binding of progesterone receptor to its target DNA without being retained in the complex (54).

HMG I and its alternatively spliced variant HMG Y (which lacks an internal stretch of 11 amino acids), are well-characterized chromosomal nonhistone proteins preferentially associated with active chromatin. These basic, low molecular weight proteins are expressed at high levels in rapidly dividing, undifferentiated, or neoplastically transformed cells. A role for HMG I/Y has been invoked in the positioning of nucleosomes on DNA (55); in the replication of ribosomal DNA (56); in the stimulation of transcription of ribosomal RNA (57) and lymphotoxin genes (58); in the virus induction of the IFN-β gene (43, 44); and in the displacement of histone H1 from scaffold attachment regions (59). Recently, HMG I/Y has been implicated in the repression of the IL-4 promoter, a finding which is in sharp contrast to its usual stimulatory effect on transcription (60). It thus appears that HMG I/Y, as a structural component of active chromatin, may serve divergent functions.

The mechanisms by which HMG I/Y can influence transcription are still incompletely understood. HMG I/Y has previously been shown to facilitate the binding of certain transcription factors to DNA (43, 44). This feature of HMG I/Y could be explained by at least two non–mutually exclusive mechanisms. First, since HMG I/Y can bend DNA, this could promote factor-binding by reducing the free energy of association of an activator with its binding site. Second, direct protein–protein interactions between HMG I/Y and transcription factors may promote the binding of the latter to their cognate DNA-binding sites. Our studies show that HMG I/Y can discriminate between octamer-binding proteins, selectively facilitating the binding of Oct-2A but not Oct-1 to the octamer site. These observations lead us to hypothesize that direct protein–protein interactions between HMG I/Y and the Oct proteins play an important role in determining the ability of HMG I/Y to facilitate binding of Oct-2A to the octamer element. Indeed, ongoing experiments in our lab indicate that HMG I/Y can interact with Oct-2A in the absence of the binding site (unpublished observations).

Our studies suggest a requirement for HMG I/Y in the induction of the DRA gene by IFN-γ. The mechanisms by which HMG I/Y may be involved in IFN-γ induction are not entirely clear at present. However, treatment of HeLa cells with IFN-γ induces the binding of a complex that contains HMG I/Y to the DRA octamer (Fig. 2 *D*). We hypothesize that IFN-γ induces posttranslational modifications in HMG I/Y that increase its affinity for the octamer element. This is reminiscent of what has been observed with the human IFN-β gene, where viral induction of gene expression results in the de novo binding of protein complexes to the promoter

which contain HMG I/Y (44). A requirement for posttranslational modification of HMG I/Y for its participation in gene activation would perhaps help explain the efficacy of antisense HMG I/Y RNA in inhibiting the induction of these two genes. During the early stages of these experiments we were surprised that antisense HMG I/Y RNA was an effective reagent in assessing the role of HMG I/Y in HLA-DRA gene transcription in view of the high abundance of both HMG I/Y RNA and protein in practically all mammalian cells. We hypothesize that much of the HMG I/Y found in the nucleus is in an inactive form, and that an extracellular signal such as IFN-γ might posttranslationally modify newly synthesized HMG I/Y to an active form. This modified HMG I/Y would be "active" because it (*a*) binds with higher affinity to DNA (as our data suggest), and/or (*b*) promotes protein–protein interactions more effectively than unmodified HMG I/Y. These possibilities are under active investigation in our laboratory.

The data presented in this study indicate that distinct (but largely overlapping) sets of transcription factors may be involved in DRA expression in different cell types. Class II–negative cell lines, such as HeLa and Jurkat, while containing most of the DNA-binding proteins known to interact with the promoter, do not have an active transcription complex assembled on the promoter, as assessed by in vivo footprinting (41). Induction with IFN-γ leads to the assembly of a transcription complex on the promoter (40). This transition could depend on the de novo synthesis or modification of critical factor(s) (which may or may not interact with DNA directly) that are critical to the formation of a functional transcription complex. Our findings suggest that HMG I/Y likely plays such a role in activation of the DRA gene by IFN-γ.

In HeLa cells induced with IFN-γ, HMG I/Y appears to function in DRA transcription via an Oct-2–independent pathway. Mutations in the octamer element have modest effects on the inducibility of the DRA promoter, while severely impairing constitutive expression in B cells. Furthermore, after induction of HeLa cells with IFN-γ, we found no evidence of de novo Oct-2 synthesis using gel-shift assays. In contrast, IFN-γ treatment enhances the binding of HMG I/Y to the octamer element. The modest effect of the octamer mutation on IFN-γ inducibility might be due to the effect of the mutation on HMG I/Y binding. The fact that double mutations in the octamer and the D box elements result in a profound reduction in IFN-γ inducibility of the promoter suggests that at least one HMG I/Y molecule bound to either of these sites is required for induction, with occupancy of both sites allowing full activation of the gene.

Apart from the mechanisms described above, HMG I/Y may also facilitate interactions between DNA-bound factors and adapter proteins (such as the recently described class II activator, CIITA; 61), or might also participate at a step before transcription complex assembly (such as alteration of chromatin structure).

In conclusion, the data presented in this paper indicate that the HMG I/Y protein and the Oct-2A transcriptional activator are essential proteins for DRA transcription. Distinct sets of transcription complexes seem to be involved in DRA expression in B cells and induced HeLa cells, and HMG I/Y appears to play a role in both situations. The addition of Oct-2A to the list of transcriptional activators facilitated by HMG I/Y further underscores the notion that HMG I/Y plays a general and critical role in transcription complex assembly.

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