Identification of CCAAT Displacement Protein (CDP/cut) as a Locus-specific Repressor of Major Histocompatibility Complex Gene Expression in Human Tumor Cells*

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The human MHC1 class I genes encode the highly polymorphic HLA class I antigen heavy chains, membrane-spanning proteins that combine with the invariant β2-microglobulin to form the HLA class I surface antigen (1). Classical MHC class I (HLA-A, -B, and -C) antigen expression is essential for processing and presentation of peptide antigens to cytotoxic CD8+ lymphocytes (2). In addition, recent evidence suggests that certain classical and nonclassical MHC I antigens play an important role in inhibition of natural killer (NK) cell function (3–6) and certain subsets of cytotoxic T-cells (7). Therefore, changes in MHC class I gene expression can have profound effects on the overall susceptibility to NK cell and cytotoxic T-cell-mediated lysis, the net effect depending on the specific MHC Class I genes involved.

Down-regulation of MHC class I gene expression is observed in many human tumors and transformed cell lines, resulting in decreased susceptibility to cytotoxic T-cell-mediated lysis (8–19). Global down-regulation of surface class I expression can arise by several mechanisms, including loss of β2-microglobulin or peptide transporter gene expression (9, 14). More frequently, loss of MHC class I antigen expression in tumor cells occurs at a single locus. For example, selective down-regulation of HLA-B locus gene expression has been observed in many cell lines derived from patients with metastatic melanoma (15) and colon cancer (16). The specific MHC class I alleles in a particular tumor that activate cytotoxic T-cells may be different from those that inhibit NK cell function. Therefore, understanding how MHC class I antigen expression is controlled at the level of specific loci and alleles is an important goal with possible implications for the development of vaccine-based cancer therapeutics.

The mechanisms that mediate locus-specific down-regulation of MHC class I gene expression are incompletely understood. Transcriptional mechanisms appear to be involved in many cases (17–19) but have not been well characterized. The promoter and 5′-flanking regions of most MHC class I genes contain several highly conserved, well characterized DNA sequence elements involved in maintenance of constitutive expression (20–22). Down-regulation of MHC class I expression due to decreased binding of members of the rel family of transcription factors to the highly conserved enhancer A element has been reported in human tumor cell lines (23) and in adenovirus-12-transformed cell lines (24). Our laboratory has previously shown that another highly conserved element, the interferon-stimulated response element, functions as a locus-specific activator of HLA-A gene expression in several

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The abbreviations used are: MHC, major histocompatibility complex; NK, natural killer; CDP, CCAAT displacement protein; CAT, chloramphenicol acetyltransferase; HSV, herpes simplex virus; th, thymidine kinase; CMV-ΔGal, β-galactosidase gene driven by the cyto-megalovirus promoter; EMSA, electrophoretic mobility shift assay; CR, cut repeat; NF-Y, nuclear factor Y; HDAC1, histone deacetylase 1;
hematopoietic tumor cell lines (25). The relative contribution of these and other positive regulatory elements in determining the steady state expression of specific MHC class I genes in normal and malignant cells remains to be elucidated.

In addition to positive regulatory elements, DNA sequence elements that mediate repression of classical MHC class I gene expression have been identified in the 5'-flanking region of some MHC class I genes, including the mouse classical class I genes H2-K and H2-L (26–31) and the swine class I gene, PD1 (32–34). Some of these elements are tissue- and/or differentiation-specific in their function. The factors that bind to most of these elements have not been fully characterized, and none of these elements has been shown to function in a locus-specific manner.

Many of the factors that bind MHC class I gene regulatory elements produce a relatively small (2–3-fold) effect on MHC class I gene expression when acting alone. However, even small changes in the level of constitutive MHC class I mRNA levels have been shown to result in much larger changes in MHC class I surface antigen expression and cytotoxic T-cell function (35–38). It is likely that the large locus-specific variations in MHC class I gene expression observed in many human tumors have been shown to result in much larger changes in MHC class I gene expression when acting alone. However, even small elements produce a relatively small (2–3-fold) effect on MHC class I gene expression.

Locus-specific Repression of MHC Class I Genes

In the current studies, we report the identification of human CDP/cut as a locus-specific (HLA-B and -C versus HLA-A) repressor of MHC class I gene expression in human tumor cells. We hypothesize that CDP/cut may contribute to the suppression of constitutive HLA-B7 gene expression observed in some MHC class I genes, including the mouse classical class I genes H2-K and H2-L (26–31) and the swine class I gene, PD1 (32–34). Some of these elements are tissue- and/or differentiation-specific in their function. The factors that bind to most of these elements have not been fully characterized, and none of these elements has been shown to function in a locus-specific manner.

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The gel-purified wild type B7 promoter PCR product was end-labeled by PCR using HLA-A2 pCATb WT as a template. The PCR corresponding region of the HLA-A2 promoter (nt 666 to 546) was generated by PCR using HLA-A2 pCATb WT as a template. The PCR products were gel-purified and used directly as specific competitors for EMSA. The gel-purified wild type B7 promoter PCR product was end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and used as a probe. Except where indicated, EMSA was carried out by incubating nuclear extract (10–20 µg of protein), 6 µM of poly(dI-dC), ATP (1 mM), 0.5 µg of guinea pig anti-CDF/cut immune serum or preimmune serum (a kind gift from Dr. Ellis Neufeld) when indicated, and specific DNA competitors, when indicated, in 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM MgCl2, 1 mM EDTA, 5% glycerol, and 0.2 mM dithiothreitol in a final volume of 20 µl. The reaction mixture was incubated for 45 min prior to the addition of probe.

Preparation of Nuclear Extracts—Nuclear protein extracts were prepared according to the method of Dignam (43) in the presence of the protease inhibitors leupeptin (10 µg/ml), pepstatin (10 µg/ml), aprotinin (10 µg/ml), and phenylmethylsulfonyl fluoride (0.5 mM). Protein concentrations in extracts were determined using a Coomassie-based protein assay (Bio-Rad).

Electrophoretic Mobility Shift Assays (EMSA)s—Double-stranded DNA fragments containing nt 666 to 547 of the HLA-B7 promoter region were generated by PCR using the following plasmids as templates: wild type HLA-B7 CAT, B7 CCAAT mutant CAT, B7 AT mutant CAT, B7 CCAAT/CAT, B7 CCAAT/CAT, B7 CCAAT/CAT, B7 CCAAT/CAT. The corresponding region of the HLA-A2 promoter (nt 666 to 546) was generated by PCR using HLA-A2 pCATb WT as a template. The PCR products were gel-purified and used directly as specific competitors for EMSA. The gel-purified wild type B7 promoter PCR product was end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and used as a probe. Except where indicated, EMSA was carried out by incubating nuclear extract (10–20 µg of protein), 6 µM of poly(dI-dC), ATP (1 mM), 0.5 µg of guinea pig anti-CDF/cut immune serum or preimmune serum (a kind gift from Dr. Ellis Neufeld) when indicated, and specific DNA competitors, when indicated, in 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM MgCl2, 1 mM EDTA, 5% glycerol, and 0.2 mM dithiothreitol in a final volume of 20 µl. The reaction mixture was incubated for 45 min prior to the addition of probe.

EMSA to detect methylcytosine-binding protein (MeCP1) binding activity in HeLa nuclear extracts was performed exactly as described (45).
The HLA-B7 Promoter Contains an Upstream Repressor Element—We initially identified two closely spaced potential binding sites for the transcriptional repressor CDP/cut in the HLA-B7 promoter, −650 nt upstream of exon I (Table I). Both of these AT-rich segments are homologous to the consensus binding site for the cut repeat 1 and 2 (CR-1 and CR-2) domains of CDP/cut (47), although only the 5′ site contains the CCAATA core sequence, which appears to be important for efficient CDP/cut binding (47, 48) (Table I). To determine whether these elements mediate repression of HLA-B7 gene expression, heterologous CAT reporter constructs containing a 664-nt fragment of the HLA-B7 promoter (Fig. 1A) were transiently transfected into HeLa cells, which express high levels of endogenous CDP/cut (42). Deletion of a 120-nt region containing the putative HLA-B7 CDP/cut binding sites (HLA-B7 del CDP construct, Fig. 1A) resulted in a statistically significant (p < 0.05), greater than 2-fold increase in reporter gene expression (Fig. 1B), demonstrating that the region of the HLA-B7 promoter contains a repressor element. Similar results were observed in K562 cells (data not shown). Constructs containing mutations of either of these AT-rich regions were still able to mediate repression of reporter gene expression (Fig. 1B). Simultaneous mutation of both of these regions resulted in a statistically significant 2-fold increase in reporter gene expression equivalent to that observed with deletion of the entire region containing the putative CDP/cut binding sites (Fig. 1, A and B).

Sequence information for the corresponding regions of other MHC class I loci and alleles is limited. A GenBank search revealed a total of only three sequence variations in the regions directly corresponding to the two potential HLA-B7 promoter CDP/cut binding sites (Table I). As shown in Fig. 1B, an HLA-B7 promoter reporter construct in which the HLA-B7 repressor element was converted to the corresponding region of the HLA-Cw2 promoter also mediated repression of reporter gene expression. In contrast to HLA-B7 and HLA-Cw2, deletion of the homologous region of the HLA-A2 promoter resulted in a significant decrease rather than an increase in expression (Fig. 1B, upper portion), demonstrating that this element represses MHC class I gene expression in a locus-specific manner.

To determine whether the HLA-B7 repressor element functions when stably integrated into chromosomal DNA, the wild type HLA-B7 CAT and HLA-B7 del CDP CAT constructs were stably transfected into K562 cells. Deletion of the HLA-B7 repressor element resulted in ~3-fold higher CAT activity (when corrected for gene copy number) compared with the wild type HLA-B7 CAT construct (data not shown).

Binding of CDP/cut to the HLA-B7 Repressor Element Is Locus-specific—A double-stranded DNA probe containing the HLA-B7 repressor element identified in Fig. 1 was used in EMSA to determine whether any specific binding activity was associated with this element. When this probe was incubated with nuclear extracts from HeLa cells (Fig. 2), a specific, low mobility complex was formed. An excess of unlabeled probe (Fig. 2) effectively competed with the wild type probe for binding. Analogous to the results obtained in the functional studies (Fig. 1), specific DNA competitors that contained mutations in either the upstream CCAAT box or the downstream AT-rich sequence were able to partially compete with the wild type binding activity.

### Table I

| Gene   | Putative CDP/cut binding site sequences (47) | CR-1 | CR-2 |
|--------|--------------------------------------------|------|------|
| HLA-B7 | CCAATAATCCGAT (A/T) (A/C/T)CC(G/A)ATA(A/G/T)(C/T)(C/G/T)(A/C/G)(A/G/T) |      |      |
| HLA-B2 | TACCAAATATT CR-1 consensus site            |      |      |
| HLA-B7 | TACCAATAATT CR-2 consensus site            |      |      |
| HLA-B7 | TACCATATT GAATTGAACAA                    |      |      |
| HLA-B7 | TACCAATTATT GAATTGAACAA                    |      |      |
| HLA-B7 | TACCAATTATT GAATTGAACAA                    |      |      |
| HLA-B7 | TACCAATTATT GAATTGAACAA                    |      |      |

Fig. 1. HLA-B and -C promoters contain a locus-specific repressor element located 650 nt upstream of exon I. A: HLA-B7 promoter reporter constructs based on the promoterless CAT reporter plasmid pCATBasic. Nucleotide positions are numbered relative to the beginning of HLA-B7 exon 1. The regions of the wild type HLA-B7 promoter that are homologous to the CDP/cut CR-2 consensus binding site (Table I) are shown in boldface type. Nucleotides that differ from the wild type HLA-B7 promoter are underlined (Fig. 1A). B: transient transfections of the HLA-B7CAT constructs in A were carried out in HeLa cells as described under “Experimental Procedures.” The plasmid HLA-A2 WT pCATb contains 687 bp of the HLA-A2 promoter. HLA-A2 del-CDP pCATb contains 525 bp of the HLA-A2 promoter (the potential CDP/cut binding sites have been deleted, analogous to the B7 del CDP construct). In the plasmid B7 Cw2 mutant pCATb, the HLA-B7 repressor element has been converted to the corresponding sequence in the HLA-Cw2 promoter (see Table I). Results, normalized to β-galactosidase activity, are reported as the mean of at least three independent experiments. Error bars indicate S.E. values for each set of experiments. Asterisks indicate values that are significantly different from the HLA-B7 WT construct (p < 0.05).
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The addition of a polyclonal guinea pig antibody to human CDP/cut resulted in complete ablation of the specific complex (Fig. 4), demonstrating that the protein complex that binds to the HLA-B7 repressor element contains human CDP/cut. Preimmune serum from the same animal had no effect on CDP/cut binding to the HLA-B7 repressor element (Fig. 4).

Overexpression of CDP/cut in a Cell Line Deficient in CDP/cut Represses the HLA-B7 Promoter, but Not the HLA-A2 Promoter—To further establish that CDP/cut represses HLA-B7 expression by binding to the HLA-B7 repressor element, the HLA-B7 CAT and the B7 del-CDP CAT reporter constructs were cotransfected with a full-length CDP/cut expression vector in CDP/cut-deficient Panc-1 cells (49). EMSA was performed on nuclear extract prepared from Panc-1 cells to confirm decreased CDP/cut DNA-binding activity in these cells (Fig. 5A). The empty expression vector was cotransfected as a control. As shown in Fig. 5B, overexpression of CDP/cut resulted in a 3.5-fold suppression of HLA-B7 promoter reporter gene expression, whereas CDP/cut overexpression had no effect on reporter gene expression when the CDP/cut binding sites were deleted from the construct. In the absence of CDP/cut expression, the HLA-B7 repressor element did not mediate repression of the HLA-B7 promoter in CDP/cut-deficient Panc-1 cells (Fig. 5B, compare the two experiments with the empty expression vector PMT2 only). Finally, overexpression of CDP/cut did not repress a reporter construct containing the corresponding region of the HLA-A2 promoter (Fig. 5B). Similar experiments were attempted in CDP/cut-deficient JY cells, but CDP/cut overexpression resulted in unacceptable toxicity in these cells (data not shown).

The HLA-B7 CDP/cut Binding Site Represses Activated Transcription from a Non-MHC Class I Promoter—To determine whether binding of CDP/cut to the HLA-B7 repressor element is able to repress transcription from a promoter other than the HLA-B7 or HLA-Cw2 promoters, the HLA-B7 CDP/cut binding site was inserted 800 nt upstream of a CAT reporter construct containing 156 nt of the HSV tk promoter (Fig. 6).

Overexpression of CDP/cut repressed expression of this construct nearly 6-fold in HeLa cells relative to the wild type HSV tk promoter reporter construct (Fig. 6). Similar experiments in the CDP/cut-deficient cell lines Panc-1 and JY were not successful due to extremely low basal expression of constructs driven by the HSV tk promoter (data not shown).

Replication of HLA-B7 Gene Expression by CDP/cut Is Not Mediated by Displacement of the Transcriptional Activator NF-Y and Does Not Involve Association of CDP/cut with HDAC1—Previous studies have shown that CDP/cut represses transcription by some promoters by displacing the positive regulatory factor NF-Y, which binds CCAAT box elements (50–52). To determine whether NF-Y binds the HLA-B7 repressor element, nuclear extracts from JY cells, a lymphoblastoid B-cell line with decreased expression of CDP/cut (Fig. 5A), were used in EMSA with an HLA-B7 repressor element probe. EMSA was carried out under conditions that have been shown to promote NF-Y binding (44). As shown in Fig. 7A, the addition of goat polyclonal antibodies to NF-Y subunits A, B, and C did not ablate or supershift any HLA-B7 repressor element binding complexes, suggesting that this element does not bind NF-Y. As a positive control, a double-stranded oligonucleotide containing an NF-Y consensus binding site was incubated with the same JY nuclear extracts (Fig. 7B), demonstrating the ability of this assay to detect NF-Y binding activity in these extracts.

Results from recent studies suggest that CDP/cut may actively repress expression of some genes by associating with the histone deacetylase HDAC1 (49, 53). To determine whether CDP/cut is associated with HDAC1 in the HLA-B7 repressor element binding complex, EMSA was carried out with HeLa nuclear extracts in which HDAC1 had been immunodepleted. As shown in Fig. 8A, depletion of HDAC1 had no effect on the formation or mobility of the CDP/cut-containing complex. Depletion of HDAC1 from the extracts was confirmed by demonstrating inhibition of MeCP1 complex formation (Fig. 8B). Association of MeCP1 with HDAC1 is required for MeCP1 to bind DNA (46). Moreover, treatment of HeLa cells with the histone deacetylase inhibitor trichostatin A (in experiments analogous to those shown in Fig. 1B) failed to relieve repression of HLA-B7 wild type reporter gene expression (data not shown).

DISCUSSION

In these studies, we have identified what is to our knowledge the first example of a locus-specific (HLA-B and -C versus HLA-A) repressor element in an MHC class I gene promoter. These experiments also identify the protein that binds to this novel repressor element as CDP/cut, the mammalian homologue of the Drosophila protein cut (54). CDP/cut overexpression in Panc-1 cells results in a 3.5-fold repression of the HLA-B7 promoter (Fig. 5B). This degree of repression is similar to that reported for several other genes containing one or two CDP/cut binding sites (55–57). CDP/cut has not been shown previously to function in a locus or allele-specific manner. While complete sequence information in this region of the promoter is available for only a few MHC class I genes, many of the alleles in Table I occur in vivo with high frequency. Therefore, it is likely that the most frequently occurring HLA-B and C alleles are susceptible to repression by CDP/cut, whereas most HLA-A alleles and nonclassical MHC class I genes are not. In contrast, none of the murine MHC class I genes (H2-D, H2-K, and H2-L) contain any regions that are homologous to the HLA-B7 repressor element.

CDP/cut is a ubiquitous transcriptional repressor first identified as a negative regulator of the sperm-specific sea urchin histone H2-B-1 gene (58). Since then, CDP/cut has been shown
CDP/cut overexpression may down-regulate the expression of several developmentally important genes during malignant transformation. CDP/cut contains a distinctive homeodomain and three highly conserved CRs. Each of the three CRs and the homeodomain of CDP/cut are able to bind DNA independently with broad binding specificities that are distinct but overlapping (47, 69). While the overall consensus binding sequence contains significant variability, the CCAATA core has been shown to be important for efficient CDP/cut binding (47, 48). As shown in Table I, the HLA-B7 repressor element contains two closely spaced regions which are homologous to the consensus binding sequences for the CR-1 and CR-2 domains of CDP/cut (47, 48). The EMSA and immunoablation experiments in Figs. 2–4 demonstrate that CDP/cut binds selectively to this region upstream of HLA-B and C promoters. Both the CCAAT and TATT motifs in the HLA-B7 promoter must be altered simultaneously to disrupt CDP/cut binding and to relieve transcriptional repression (Figs. 1 and 2), suggesting that CDP/cut-binding to the HLA-B7 repressor occurs through either site. In the HLA-A2 promoter, both the CCAAT and TATT motifs contain sequence variations that have been shown to disrupt binding of CDP/cut to other targets (47, 48). Although the 5’ CCAAT motif of the HLA-A2 promoter differs from the HLA-B7 promoter CCAAT motif by only one base pair (Table I), the mutation alters the important CCAATA core sequence. This sequence variation significantly interferes with CDP/cut binding to the HLA-A2 promoter (Figs. 3, B and D) and results in the inability of CDP/cut to repress expression from the HLA-A2 promoter (Figs. 1B and 5B). In contrast, the corresponding region of the HLA-Cw2 promoter contains two CAATA motifs, consistent with the apparent higher affinity of this site for CDP/cut (Fig. 3C). A recent study demonstrated that cooperative binding of isolated recombinant CDP/cut CR-1 and CR-2 domains is enhanced by the presence of two juxtaposed CAAT sites, compared with a single site (48). The same study also showed that the ATCAAT motif, present in the HLA-Cw2 promoter (Table I), is a high affinity site for cooperative binding of the CDP/cut CR-1 and HD domains. Despite increased affinity of the HLA-
Cw2 promoter for CDP/cut compared with the HLA-B7 promoter, both of these elements repress reporter gene expression to a similar extent (Fig. 1B). Since the degree of repression observed with either element is relatively small, it is possible that any difference may not be detectable. Alternatively, maximal repression may occur only when one CDP/cut site is occupied due to interference from a closely bound second CDP/cut molecule at the adjacent site or due to conformational restraints resulting from a single CDP/cut molecule bound at both sites.

The mechanism of transcriptional repression of HLA-B and -C locus gene expression by CDP/cut remains to be elucidated. CDP/cut was initially identified as a protein that directly displaces the transcriptional activator CCAAT-binding protein in the histone H2-B-1 gene (58) and the gp91-phox gene (59). In the immunoglobulin heavy chain gene, CDP/cut represses transcription by displacing the B-cell-specific Bright transcription factor from its binding site in the intronic enhancer (67). Displacement of the CCAAT-binding transcriptional activator NF-Y by CDP/cut has been demonstrated for a few genes (50, 52). In CDP/cut-deficient Panc-1 cells, the HLA-B7 repressor element functions as a weak activator of the HLA-B7 promoter (see Fig. 5B, experiments with the empty expression vector PMT2), which suggests that CDP/cut may be displacing a positive transcriptional activator from an overlapping binding site. However, our EMSA results using nuclear extracts that contain NF-Y, but very little CDP/cut, suggest that NF-Y does not bind the HLA-B7 repressor element (Fig. 7). A TRANSFAC analysis (70) using the 32-nt HLA-B7 and Cw2 repressor elements failed to identify other known transcription factors that could potentially bind these regions. Therefore, if CDP/cut represses HLA-B and -C gene expression by displacement of positive regulatory factors, the identity of these factors remains unknown. Alternatively, the 120-nt DNA sequence used in the reporter constructs in this study may contain an unidentified positive regulatory element for which activity is only observed in the absence of CDP/cut expression.

Other evidence suggests that the C-terminal domain of CDP/cut functions at a distance as a direct transcriptional repressor of some genes (71), possibly by association with histone deacetylase activity (49, 53). However, our results indicate that neither DNA binding nor transcriptional repression of HLA-B7 gene expression by CDP/cut appear to require HDAC1. Taken together, these observations suggest that CDP/cut represses transcription by multiple, promoter-specific mechanisms. Whatever the mechanism, transcriptional repression mediated by CDP/cut binding to the HLA-B or -C repressor elements does not appear to involve interactions that are specific for MHC class I promoters. As seen in Fig. 6, activated transcription driven by the HSV tk promoter is repressed nearly 6-fold when the HLA-B7 repressor element is inserted upstream of the tk promoter.
While MHC class I repressor elements have been previously described, none of these elements are locus-specific. Only one of these elements, a silencer identified in the miniature swine class I gene PD1, is homologous to the HLA-B locus repressor element (34). The PD1 silencer element was among the first negative elements to be described in an MHC class I gene. Given the similarity of the swine PD1 silencer element to the human HLA-B repressor element, it is possible that swine CDP/cut also binds the PD1 silencer element.

Most reported regulatory factors have a relatively small effect on MHC class I gene transcription, depending on the cell type and the specific MHC class I locus (25, 72–74). However, even small changes in MHC class I gene expression (i.e. 2-fold) can have a profound effect on MHC class I antigen cell surface expression and the resultant effect on cytotoxic T-cell function (35–38). For this reason, it is likely that overexpression of CDP/cut in tumor cells, which produces up to a 3.5-fold decrease in expression driven by the HLA-B7 promoter (Fig. 5B), has important consequences on MHC class I antigen surface expression.

In summary, we have identified a repressor element that mediates locus-specific down-regulation of HLA-B and -C (versus HLA-A) gene expression in human tumor cell lines. We have also shown that the transcriptional repressor CDP/cut binds this element in a locus-specific manner and mediates the observed repression. It is interesting to speculate that overexpression of CDP/cut contributes to the frequent observation of selective HLA-B locus down-regulation in melanoma, colon cancer, and other human tumors. Understanding the mechanisms that control locus-specific regulation of MHC class I gene expression has potentially important implications for the development of vaccine-based cancer therapeutics.

**Fig. 7.** NF-Y does not bind the HLA-B7 repressor element. A, EMSA was carried out using JY nuclear extracts (which contain reduced levels of CDP/cut) under conditions that promote binding of NF-Y (see “Experimental Procedures”). Polyclonal antibodies to NF-Y subunits or preimmune polyclonal IgG were added as indicated. B, EMSA was carried out as in Fig. 7A except that an NF-Y consensus binding site probe was used.

**Fig. 8.** CDP/cut does not associate with HDAC1 when bound to the HLA-B7 repressor element. A, HeLa nuclear extract was depleted of HDAC1 by incubation with agarose-conjugated HDAC1 antibody or treated with agarose-conjugated goat preimmune IgG. EMSA was carried out using a wild type HLA-B7 repressor element probe. B, HeLa nuclear extract, treated as in A, was used in EMSA with a methylated MeCG11 probe. The binding of MeCP1 to MeCG11 has been previously shown to require association with HDAC1 (see “Results”), confirming depletion of HDAC1 in the nuclear extracts.
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