The SANT Domain of Human MI-ER1 Interacts with Sp1 to Interfere with GC Box Recognition and Repress Transcription from Its Own Promoter*

Zhihu Ding, Laura L. Gillespie, F. Corinne Mercer, and Gary D. Paterno‡

From the Terry Fox Cancer Research Laboratories, Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John’s, Newfoundland A1B 3V6, Canada

Received for publication, April 5, 2004
Published, JBC Papers in Press, April 26, 2004, DOI 10.1074/jbc.M403793200

The SANT domain of human MI-ER1 (human mesoderm induction-early response 1) is a growth factor-induced immediate early gene encoding a novel transcriptional regulator (1, 2) that is differentially expressed in breast carcinoma cell lines and tumors (3).

hmi-er1 is transcribed from two distinct promoters P1 and P2 (4), and transcripts undergo alternative splicing to produce six protein isoforms differing in their N and C termini (4). Two of the N-terminal variants differ in the sequence of their 5'-UTR, whereas the third contains an additional exon that encodes 25 amino acids (aa). The C-terminal variants, hMI-ER1α and -β, differ both in the size and sequence of their C-terminal domains: the α C terminus consists of 23 aa and includes an LXXLL motif, a domain known to be important for interaction with nuclear hormone receptors (5). In this regard, the α isoform mRNA is only detectable in endocrine tissues (4). The β C terminus contains 102 aa and includes the only functional nuclear localization signal (4, 6). Whereas the divergent C-terminal amino acid sequences would suggest that these two isoforms have distinct functions, so far no difference in function has been determined. Instead, both isoforms can act as transcriptional repressors, and this repression was shown to involve recruitment of histone deacetylase 1 (HDAC1) (2).

The common internal sequence of hMI-ER1 contains conserved domains found in a number of transcriptional regulators, including an acid activation domain (1), an ELM2 domain (7), and a signature SANT domain (8). In a recent report, we showed that the ELM2 domain functions in the recruitment of HDAC1 and transcriptional repression (2). The SANT domain is located immediately downstream of the ELM2 domain and, in other proteins, has been implicated in DNA binding as well as in protein-protein interactions (8), including interactions with histone deacetylase 3 (HDAC3)- and histone acetyltransferase-containing complexes (9–11), as well as nonacetylated histones (12). Recently, the SANT domain of the nuclear receptor corepressor SMRT has been implicated in the ability of this protein to target specific promoters through interpretation of the so-called histone code (12). To date, no function has been ascribed to the SANT domain of hMI-ER1.

In this report, we investigate mechanisms of transcriptional regulation of the hmi-er1 P2 promoter and demonstrate that promoter activity is regulated by Sp1 protein and that this activation is repressed in a dose-dependent manner by hMI-ER1 protein. This autorepression does not depend upon HDAC activity but involves interference of Sp1 binding to the chromatin surrounding the hmi-er1 P2 promoter and from the cognate binding sites by physical association with a region

* This work was supported by Grant MT-15378 from the Canadian Institutes for Health Research (L. L. G. and G. D. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Terry Fox Cancer Research Laboratories, Division of Basic Medical Sciences, Faculty of Medicine, Memorial University, 300 Prince Phillip Dr., St. John’s, Newfoundland A1B 3V6, Canada. Tel: 709-777-7012; Fax: 709-777-7010; E-mail: gpaterno@mun.ca.

hmi-er1 (human mesoderm induction-early response 1) is a growth factor-induced immediate early gene encoding a novel transcriptional regulator.

The abbreviations used are: UTR, untranslated region; aa, amino acid; ChIP, chromatin immunoprecipitation; DTBP, 3-3-pidithiobispropiomimidate-2HCl; EMSA, electrophoretic mobility shift assay; GST, glutathione S-transferase; HDAC, histone deacetylase; TSA, trichostatin A; RLU, relative luciferase units; IP, immunoprecipitation; dox, doxcycline.
containing the hMI-ER1 SANT domain. This represents a novel mechanism for negative regulation of Sp1 target promoters and a functional role for the SANT domain in the activity of co-repressor regulatory factors.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—All cell lines were obtained from the American Type Culture Collection and cultured at 37 °C in 5% CO₂ in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum.

**Plasmids and Constructs**—A 1460-bp sequence, containing 144 bp of hmi-er-1 5'-UTR and 1316 bp of 5'-flanking genomic sequence, was generated by PCR from human genomic DNA, using the primer pairs listed in Table I. The PCR product (1316) was cloned into the pCR2.1 vector (Invitrogen), sequenced on both strands, and subcloned into the BamHI/HindIII sites of the plasmid pGL3-Basic vector (Promega Corp.). The plasmid pGL3(AS) was generated by digestion of pCR(1316) with SmaI digestion and subcloning into the KpnI/XhoI sites of pGL3-Basic. The pGL3(Δ1316) plasmid was generated from pGL3(AS) by digestion of pCR(Δ1316) with EcoRI and subcloning into the XhoI/HindIII sites of pGL3-Basic.

**Transactivation Assays**—All transfections and TSA treatments were performed as previously described (2), in duplicate in 6-well plates, using the indicated amount of plasmid DNA, and cells were harvested after 48 h in culture. Luciferase assays were performed on cell lysates using a Monolight 2010 Luminometer (Analytical Luminescence Laboratory) and a luciferase assay reagent (Promega), according to the manufacturer’s directions. The values obtained were normalized to the number of RLU.

**Electrophoretic Mobility Shift Assays**—Electrophoretic mobility shift assays (EMSA) were performed as described (13). Briefly, the double-stranded consensus Sp1 oligonucleotides (Promega) or the minimal functional promoter fragment (−68 to +144) was labeled with [γ-32P]ATP and T4 polynucleotide kinase and purified on NucTrap probe purification columns (Stratagene, Inc.). The labeled double-stranded oligonucleotides were incubated with 2 μl of HeLa nuclear extract (Promega) or GST fusion protein at room temperature for 20 min in 20 μl of reaction buffer containing 5% glycerol, 5 mM MgCl₂, 1 mM dithiothreitol, 50 mM KCl, 10 mM ZnSO₄, 85 μM bovine serum albumin, and 50 mM HEPES (pH 7.5). Poly(dI-dC) was used as a heterologous competitor in the reaction (2 μg/reaction). Where indicated, a 20-fold molar excess of unlabeled probe was included in the binding reaction. For antibody supershift assays, the extract was incubated for 30 min at room temperature with Sp1-specific antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Bound and free probes were resolved by nondenaturing electrophoresis on 4% polyacrylamide gels and analyzed by autoradiography.

**GST Fusion Protein Production**—GST fusion proteins were expressed in Escherichia coli BL21 and purified according to the instructions supplied with the pGEX-4T-1 and pGEX-4T-2 vectors. GST fusion protein level and purity were determined by SDS-PAGE.

**Electroporation (Co-IP), GST Pull-down Assays and Western Blot Analysis**—Coupled transcription-translation (TNT, Promega) reactions and in vitro co-IP assays were performed as described in Ding et al. (2); the antibody used was either an anti-Sp1 antibody (Santa Cruz Biotechnology) or the anti-Myc monoclonal antibody, 9E10 (a kind gift from Dr. K. Kao, Memorial University). Pull-down assays with GST fusion proteins were performed as described in Ref. 14, using 1 μg of GST fusion protein, 50 μl of glutathione-Sepharose beads (Amersham Biosciences) and 50,000 cpm of 32P-labeled TNT product. Bound proteins were analyzed by SDS-PAGE and autoradiography. For all assays, one-twentieth volume of the indicated TNT was loaded into the input lanes.

**Establishment of Stable hMI-ER1a or -b Tet-On HeLa Cell Lines and Doxycycline Induction**—hMI-ER1a and b Tet-On HeLa cell lines were generated using the hmi-er-1a or -b coding region inserted into the

---

**Table I**

| Construct | Forward primer | Reverse primer |
|-----------|----------------|----------------|
| PGL3(−1316) | 5'-GACTGTCTGTAACGTTCCTTTCC-3' | 5'-CGTACTGGCGGTGCTACATCC-3' |
| PGL3(−945) | 5'-GACTGTCTGTAACGTTCCTTTCC-3' | 5'-CGTACTGGCGGTGCTACATCC-3' |
| PGL3(−129) | 5'-CTGGACCTCGATTCGTCGGC-3' | 5'-CGTACTGGCGGTGCTACATCC-3' |
| PGL3(−68) | 5'-AGTTACAGTGTGACCTCAGG-3' | 5'-CGTACTGGCGGTGCTACATCC-3' |
| PGL3(+28) | 5'-AGTTACAGTGTGACCTCAGG-3' | 5'-CGTACTGGCGGTGCTACATCC-3' |
| hmi-er-1α | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-CGGGAGCTCTTACTCTGTGTTTTCAAG-3' |
| aa 1–512 | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-ATCTCCCTACTCTGTGTTTTCAAC-3' |
| aa 1–283 | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-CGGGATCCAAAACAGACACACGAGG-3' |
| aa 283–433 | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-CGGGATCCAAAACAGACACACGAGG-3' |
| aa 287–410 | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-CGGGATCCAAAACAGACACACGAGG-3' |
| aa 287–357 | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-CGGGATCCAAAACAGACACACGAGG-3' |
| aa 287–329 | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-CGGGATCCAAAACAGACACACGAGG-3' |
| aa 287–512 | 5'-CACCTAGGGCACATCCTGTAAC-3' | 5'-CTGACTGCCGGGTCACATCTCC-3' |

* Deletion constructs were named according to the encoded aa residues of the hMI-ER1a or -b protein.
pTRE2 vector and a Tet-On gene expression system (Clontech), according to the manufacturer’s protocol. Control cell lines were generated by transfection with the pTRE2 empty vector. Stable clones were induced to express hMI-ER1 or β using 2 μg/ml doxycycline (dox), and expression was verified by Western blot analysis of whole cell extracts, using an anti-hMI-ER1 antibody (4).

Quantitative Real Time PCR and Semiquantitative PCR Analysis—RNA was extracted from uninduced and dox-induced Tet-On cell lines and reverse transcribed as in Ref. 1. Quantitative real time PCR was performed using the Syber Green PCR Master Mix and the ABI Prism 7000 SDS (Applied Biosystems), according to the manufacturer’s protocol. The 5′-UTR of hmi-er1 was amplified using 5′-AGTGCCGGC-GGAGCCCGAGA-3′ (forward) and 5′-CTGACTGCGGGTGTCATC-CTCC-3′ (reverse), whereas β-actin was amplified using 5′-ATCTGGG-ACCCACACCTCTCTACAGGAGGCTGCC-3′ (P) and 5′-ATGCCGCGGTGTGGAGTCTC-3′ (R). Data analysis and the ratio of expression in induced cells relative to uninduced cells (relative expression ratio) was calculated as described in Ref. 15. Semiquantitative PCR was performed as in Ref. 4 using the primers listed above to amplify β-actin and the 5′-UTR of hmi-er1, as well as 5′-CAAGGGCTGAAGGCCTATGG-3′ (forward) and 5′-CAAAATCGTTGTGTGCTGAGC-3′ (reverse) to amplify the coding region of hmi-er1.

Chromatin Immunoprecipitation Assays—Chromatin from HeLa cells or dox-induced Tet-On cell lines was cross-linked with formaldehyde, sheared, and then subjected to immunoprecipitation as described by Weinmann et al. (16), with the following modifications. Anti-Sp1 (Santa Cruz) or anti-pan-hMI-ER1 (4) antibodies were used for immunoprecipitation, and 50 μl of Protein A-Sepharose (Amersham Biosciences) was used instead of Staph A cells. Immunoprecipitated chromatin was eluted, treated with RNase A, and then treated with proteinase K as in Ref. 16. PCR was performed as in Ref. 1 using 5 μl of resuspended DNA and 30 cycles, with 5′-TTTCTCTCTGCTGTGTA-ATGC-3′ and 5′-GAGATGTGACCCGGCAGTAC-3′ as forward and reverse primers, respectively. For experiments using 3′3′-dithiodisopro-linomide-2HCl (DTBP), cells were treated for 30 min with 5 μl DTBP, as described in Ref. 17, prior to cross-linking with formaldehyde.

RESULTS

Cloning of hmi-er1 5′ Regulatory Sequence and Identification of the Minimal Functional Promoter—A genomic DNA fragment encompassing the promoter region, P2 (4), and containing 1460 bp upstream of the ATG translational start codon of hmi-er1 was isolated by PCR from human genomic DNA. This sequence contains 144 bp of 5′-UTR (+1 to +144) and 1316 bp (−1 to −1316) upstream of the start of transcription (4).

Computer-assisted analysis of the 1460-bp fragment to identify potential cis-regulatory elements predicted a CpG island located between nucleotide position −389 and the start of translation (Fig. 1A). Further analysis revealed that, like many other GC-rich promoters, the upstream sequence does not contain a TATA box; nor does it contain an initiator (18) or a downstream promoter element (19). A number of potential transcription factor binding sites, including multiple Sp1 binding sites, were identified using TFSEARCH, PROSCAN, and TESS (Fig. 1A).

The 1460-bp fragment was subcloned into the promoterless luciferase reporter vector, pGL3-Basic, in sense and antisense orientations, and its promoter activity was analyzed in HeLa cell lines and ranged from 10- to 34-fold higher than control SK-OV-3, and U87. Luciferase activity was high in all transfected cell lines and ranged from 10- to 34-fold higher than control levels, demonstrating that the hmi-er1 promoter can function in a variety of cell types (data not shown).

To identify the minimal functional region of the hmi-er1 promoter, a series of 5′ deletions was constructed in pGL3 and transiently transfected into HeLa cells. Deletion of nucleotides −1316 to −133 resulted in little change in luciferase activity (Fig. 1B). Further deletion to nucleotide −68 reduced but did not abolish luciferase activity, whereas deletion to nucleotide +28 completely abolished activity (Fig. 1B). These data dem-

![Image](https://via.placeholder.com/150)
The hmi-er1 minimal promoter is predicted to contain four Sp1 binding sites (Fig. 1A); therefore, we investigated whether Sp1 binds to this sequence. EMSAs were performed using a HeLa cell nuclear extract and a probe consisting of the minimal promoter sequence (−68/+144). Two bands representing DNA-protein complexes appeared in samples containing nuclear extract (Fig. 1C, lane 2); however, only the larger complex was specific, as revealed by competition with excess unlabeled probe (Fig. 1C, lane 3). Supershift assays, using an Sp1 antibody that does not bind to this sequence (Fig. 1C, lane 2), revealed that repression is HDAC-independent. HeLa cells were co-transfected with pGL3(−68) and either pMyc, pMyc-α, or pMyc-β and then cultured in the presence or absence of the indicated amount of TSA. Cells were harvested 48 h after transfection, and the RLU for each sample was determined as described in the legend to Fig. 1. Shown are the average values and S.D. for three independent experiments. The amount of Myc-α or Myc-β protein present in each sample was determined by Western blot, using an anti-Myc antibody, and representative blots are shown.

We have shown previously that hMI-ER1 functions as a HDAC-dependent transcriptional repressor (2); therefore, we investigated whether hMI-ER1 could repress transcription from its own promoter. HeLa cells were co-transfected with pGL3(−68) along with a plasmid expressing Myc tag alone (pMyc) or fused to hMI-ER1 (pMyc-α) or hMI-ER1β (pMyc-β). Myc-α and Myc-β repressed the activity of the hmi-er1 minimal promoter to 40 and 33% of control, respectively (Fig. 2B). The HDAC dependence of this repression was determined by assay.
An obvious question is whether hMI-ER1α and -β can affect transcriptional activation by Sp1. To investigate this, HeLa cells were co-transfected with pGL3(-68), pCR-Sp1, and increasing concentrations of pMyc, pMyc-α, or pMyc-β. Promoter activation by Sp1 was repressed in a dose-dependent manner by both hMI-ER1α and -β (Fig. 3), and repression resulted in a 50% reduction in luciferase activity at the highest hMI-ER1 levels. This repression was not due to a down-regulation of Sp1 protein, which remained constant at all hMI-ER1 levels (Fig. 3).

We have confirmed that this repression also occurs at the level of the endogenous hmi-er1 gene, using dox-inducible hMI-ER1 HeLa cell lines (Fig. 4A). We treated hMI-ER1α-expressing (HTα222), hMI-ER1β-expressing (HTβ53), and control (HTC314) cell lines with 2 μg/ml dox to induce hMI-ER1 expression and then extracted RNA for real time RT-PCR and for semiquantitative RT-PCR analysis. We compared changes in the steady state levels of endogenous hmi-er1 mRNA using primers in the noncoding region of hmi-er1 that are absent in the transfected construct. Fig. 4B shows that dox-induced expression of hMI-ER1 reduced steady state levels of endogenous hmi-er1 mRNA by 50% in HTα222 cells and 42% in HTβ53 cells, relative to the expression level in HTC314 cells. This level of repression is consistent with the level of repression by hMI-ER1 observed in transient transfection approaches reported in Fig. 3A. A similar reduction in the expression level of endogenous hmi-er1 mRNA was observed using semiquantitative PCR (Fig. 4C). As expected, amplification using primers in the coding region revealed an increase in the total hmi-er1 mRNA expression level, due to dox induction of the transfected hmi-er1 cDNA, whereas no discernable effect of the steady state levels of endogenous β-actin was observed (Fig. 4C).

hMI-ER1 Is Associated with the Chromatin of Its Own Promoter and Interferes with Sp1 Binding—Although we have demonstrated that Sp1 can bind to recognition sequences in the hmi-er1 promoter, it is important to demonstrate that endogenous Sp1 can associate with the hmi-er1 promoter in vivo. Therefore, we performed ChIP assays using HeLa cells to investigate this possibility. Formaldehyde-cross-linked, sheared chromatin was isolated and subjected to immunoprecipitation using an anti-Sp1 antibody or nonimmune serum. Following purification and reversal of the cross-links, the DNA was amplified using primer sets flank ing the minimal hmi-er1 promoter. Fig. 5A shows that the hmi-er1 promoter could be am-
plified from chromatin immunoprecipitated with anti-Sp1 (lane 1) but not with nonimmune serum (lane 2). Next ChIP assays were performed to determine whether hMI-ER1 was associated with the chromatin of the endogenous hmi-er1 promoter region. HTβ53 cells were induced with 2 μg/ml dox and then fixed with either formaldehyde alone or with an additional protein–protein cross-linking step using DTBP, followed by formaldehyde. Sheared chromatin was isolated and subjected to ChIP using a pan-hMI-ER1 antibody (4). As can be seen in Fig. 5B, DNA sequence encompassing the hmi-er1 promoter co-immunoprecipitated with the hMI-ER1 protein, but only in the presence of DTBP (compare lanes 3 and 7). The specificity of this interaction was confirmed using preimmune serum (lanes 2 and 6). The failure to cross-link hMI-ER1 protein to its promoter with formaldehyde alone is not a technical difficulty, since Sp1 was always associated with the promoter under similar conditions (see Fig. 5A, lane 1). Finally, we utilized ChIP analysis to determine whether there was an interaction between hMI-ER1 and Sp1 proteins at the level of the chromatin. We isolated formaldehyde cross-linked chromatin from dox-induced HTβ53 cells and HTC314 control cells and subjected it to immunoprecipitation with Sp1 or nonimmune antibodies, followed by amplification of the DNA from the hmi-er1 promoter region. The results in Fig. 5C demonstrate that Sp1 protein was associated with the hmi-er1 promoter in control cells (lane 1) but not in cells expressing elevated levels of hMI-ER1 protein (lane 5). Taken together, these results demonstrate that both hMI-ER1 and Sp1 are associated with the chromatin in the hmi-er1 promoter and that hMI-ER1 interferes with Sp1 binding to the endogenous hmi-er1 promoter.

hMI-ER1α and -β Do Not Bind to DNA but Physically Interact with Sp1 in Vitro and in Vivo—There are several possible mechanisms by which hMI-ER1 could interfere with Sp1 binding to the promoter. One possibility is that hMI-ER1 binds to specific DNA sites in the hmi-er1 promoter. The SANT domain present in hMI-ER1 is related to the DNA binding domain of the c-myb proto-oncogene (8), and therefore we investigated the possibility that hMI-ER1 binds to DNA sequences in its own promoter using EMSAs. EMSAs were performed using a probe containing the minimal promoter sequence (−68/+144) and purified GST-Sp1, GST-hMI-ER1α (GST-α), or β (GST-β), in the presence or absence of excess unlabeled probe. As shown in Fig. 6A, GST-Sp1 binds specifically to the minimal promoter, whereas GST-α and GST-β do not. These results are in agreement with our previous results, which could not identify a consensus DNA binding site for hMI-ER1 using CASTing techniques (2), and argue against hMI-ER1 binding to cis-acting elements as a possible mechanism for repression of its own promoter. These data in combination with the results of our ChIP assays suggest that hMI-ER1 is not bound to DNA but to protein component(s) of the chromatin containing the endogenous hmi-er1 promoter.

Next we examined whether this was a direct interaction between hMI-ER1 and Sp1 proteins by investigating the ability of hMI-ER1α and -β isoforms to physically associate with Sp1, using co-immunoprecipitation assays. 35S-labeled Sp1 was synthesized in vitro, mixed with GST-α or GST-β, and then subjected to pull-down assays (Fig. 6B). Sp1 protein was detected in pull-downs with both hMI-ER1α and -β (Fig. 6B, lanes 3 and 4), but not with GST alone (Fig. 6B, lane 2).

In vivo interaction between hMI-ER1 and Sp1 was examined by transiently expressing Myc, Myc-α, or Myc-β in HeLa cells. Cell extracts were subjected to immunoprecipitation with anti-Sp1, followed by Western blotting with anti-Myc. As shown in Fig. 6C, both hMI-ER1α and -β co-immunoprecipitated with endogenous Sp1. To verify that endogenous, native complexes containing hMI-ER1 and Sp1 exist in the cell, co-immunoprecipitation analysis of extracts from nontransfected HeLa cells was performed, using preimmune serum or a pan-hMI-ER1 antibody. Sp1 protein was detected in the hMI-ER1 immunoprecipitate (Fig. 6D, lane 2), but not in the control (Fig. 6D, lane 1), demonstrating that endogenous hMI-ER1 and Sp1 proteins can physically associate in vivo.
The SANT Domain of hMI-ER1 Is Required for Interaction with Sp1—To determine which region of the hMI-ER1 protein is required for interaction with Sp1, a series of GST-α and -β deletions was constructed (Fig. 7A), and pull-down assays were performed using 35S-labeled Sp1. Our deletion analysis revealed that the sequence required for interaction with Sp1 maps to aa 287–357 (Fig. 7B), a region that contains the SANT domain (aa 288–332). Further deletion of this region at the C-terminal end to remove Lys331-Lys332 (KK), the last 2 aa of the SANT domain, also resulted in loss of Sp1–DNA complexes. EMSAs were performed as described under “Experimental Procedures,” using GST-Sp1 and 35P(–68) in the absence (lanes 1) or presence (lanes 2–6) of the indicated GST fusion protein. GST-(287–357) contains an intact SANT domain, whereas GST-(287–330) contains a truncated SANT domain, lacking the last 2 aa. The arrowhead indicates the position of specific DNA–protein complexes. D, aa 287–357 of hMI-ER1 block binding of Sp1 to the minimal hmi-er1 promoter. EMSAs were performed using GST-Sp1 and a 32P-labeled oligonucleotide (5'-ATTCCATGCCGGCCGCGGAGC-3'), representing the Sp1 consensus sequence (Sp1CS), in the absence (lanes 1 and 2) or presence (lanes 3–7) of the indicated GST fusion protein.

The transcription of a eukaryotic gene is regulated by the combined action of multiple sequence-specific transcription factors, general transcription factors, histone modifiers, cofactors, and mediators that regulate transcription factor activity and chromatin structure. Our previous studies revealed the hMI-ER1 SANT domain is required for interaction with Sp1, and that this activity is dependent upon recruitment of HDAC1 activity by the conserved ELM2 domain (2). In contrast, we show here that the activity of the hmi-er1 promoter was repressed by hMI-ER1 in an HDAC-independent manner and involved interference with Sp1 binding.

Sp1 is a sequence-specific transcription factor that binds GC and GT boxes to activate a wide range of viral and cellular genes (reviewed in Ref. 20). Sp1 is important both in transcription initiation and activation, and it can be regulated by multiple mechanisms in a cell type-specific and promoter context-specific manner (reviewed in Ref. 21). Sp1 has been linked to the maintenance of methylation-free Cpg islands (22), and the hypermethylation around Sp1 binding sites has been reported to reduce Sp1 binding, thereby decreasing transcription (23). There are several other mechanisms that serve to regulate transcription through Sp1. Transcription regulators, such as Sp3, Sp4, and BTEB3, compete with Sp1 for binding to core cis-elements and repress transcription (24, 25). Sp1 activity can also be regulated through protein–protein interactions.

**DISCUSSION**

The transcription of a eukaryotic gene is regulated by the combined action of multiple sequence-specific transcription factors, general transcription factors, histone modifiers, cofactors, and mediators that regulate transcription factor activity and chromatin structure. Our previous studies revealed the hMI-ER1 SANT domain is required for interaction with Sp1, and that this activity is dependent upon recruitment of HDAC1 activity by the conserved ELM2 domain (2). In contrast, we show here that the activity of the hmi-er1 promoter was repressed by hMI-ER1 in an HDAC-independent manner and involved interference with Sp1 binding.

Sp1 is a sequence-specific transcription factor that binds GC and GT boxes to activate a wide range of viral and cellular genes (reviewed in Ref. 20). Sp1 is important both in transcription initiation and activation, and it can be regulated by multiple mechanisms in a cell type-specific and promoter context-specific manner (reviewed in Ref. 21). Sp1 has been linked to the maintenance of methylation-free Cpg islands (22), and the hypermethylation around Sp1 binding sites has been reported to reduce Sp1 binding, thereby decreasing transcription (23). There are several other mechanisms that serve to regulate transcription through Sp1. Transcription regulators, such as Sp3, Sp4, and BTEB3, compete with Sp1 for binding to core cis-elements and repress transcription (24, 25). Sp1 activity can also be regulated through protein–protein interactions.
Factors that interact with Sp1 include E2F1, GATA1, and YY1, all of which act synergistically with Sp1 on DNA to increase transcriptional activity (26–29). Another set of Sp1-interacting transcription factors that impair Sp1-mediated transcriptional activity includes p107, PML, FB1-1, TAF-1, and MDM2 (30–34). Furthermore, HDAC1-containing complexes can be recruited directly by Sp1 for transcriptional repression (35). Whereas Rb has not been shown to bind Sp1 directly, it can increase Sp1 activity by releasing Sp1 from MDM2-Sp1 complexes (34). Post-translational modifications of Sp1, such as phosphorylation, are also critical for regulating Sp1 activity (reviewed in Ref. 21). Thus, there are several different kinds of mechanisms for regulating Sp1 transcriptional activity.

The present study showed that hMI-ER1 could form complexes with Sp1 in vitro and in vivo and that this interaction interferes with Sp1 recognition and binding to GC boxes. Thus, hMI-ER1 represses transcription of its own promoter, most likely by interfering with the chromatin association and DNA binding activity of Sp1 in vivo. This transcriptional regulatory mechanism is distinct from that reported previously for the hMI-ER1, in which repression was dependent upon TSA-sensitive HDAC1 recruitment through the ELM2 domain (2). This indicates that hMI-ER1 can function as a transcriptional repressor through both HDAC-dependent and -independent mechanisms, utilizing distinct domains. Both HDAC-dependent and -independent transcriptional repression mechanisms have been reported for other transcription regulators, such as Rb (36), Stra13 (37), and p107, p107, PML, FBI-1, TAF-1, and MDM2 (30–34). Furthermore, HDAC1-containing complexes can be re-recruited to modify the chromatin in and around the GC box in the promoter or by modifying Sp1 itself. However, our data show that hMI-ER1-mediated repression of Sp1 activity does not involve HDAC but direct binding to Sp1 itself. Together, these data reveal a novel mechanism for the negative regulation of Sp1 target promoters. The role of hMI-ER1 in regulating the expression of other cellular genes, particularly known Sp1 target genes, is currently being investigated.

Thus hMI-ER1, like other SANT-containing proteins including N-CoR, SMRT, and MTA1–3, is part of a multiprotein transcriptional regulatory complex with several critical activities, including modulating DNA binding to alter chromatin structures (2). The function of these complexes and the molecules in them is context-dependent and is determined by the presence, stoichiometry, and modifications of these regulatory molecules in different cell types to precisely regulate cellular responses and behavior. Feedback regulation of hMIER1 levels provides a precise mechanism for regulating a critical component of the transcriptional machinery.

Acknowledgments—We thank Yuan Lew and Paula Ryan for excellent technical assistance.

REFERENCES

1. Paterno, G. D., Li, Y., Luchman, H. A., Ryan, P. J., and Gillespie, L. L. (1997) J. Biol. Chem. 272, 25591–25595
2. Ding, Z., Gillespie, L. L., and Paterno, G. D. (2003) Mol. Cell. Biol. 23, 250–258
3. Paterno, G. D., Mercer, F. C., Chaytor, J. J., Yang, X., Robb, J. D., and Gillespie, L. L. (1998) Gene (Amst.) 222, 77–82
4. Paterno, G. D., Ding, Z., Lee, Y.-Y., Nah, G. N., Mercer, F. C., and Gillespie, L. L. (2002) Gene (Amst.) 295, 79–88
5. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1995) Nature 378, 733–736
6. Post, J. N., Gillespie, L. L., and Paterno, G. D. (2001) FEBS Lett. 502, 41–45
7. Solari, F., Bateman, A., and Ahinger, J. (1999) Development 126, 2483–2494
8. Ausland, R., Stewart, A. F., and Gibson, T. (1996) Trends Biochem. Sci. 21, 87–88
9. Guenthner, M. G., Barak, O., and Lazar, M. A. (2001) Mol. Cell. Biol. 21, 6091–6101
10. You, A., Tong, J. K., Grozinger, C. M., and Schreiber, S. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1454–1458
11. Sterner, D. E., Wang, X., Bloom, M. H., Simon, G. M., and Berger, S. L. (2002) J. Biol. Chem. 277, 1677–1685
12. Yu, J., Li, Y., Ishizuka, T., Guenthner, M. G., and Lazar, M. A. (2003) EMBO J. 22, 3403–3410
13. Kindle, K. W., and Vogelstein, B. (1996) Mol. Cell. Biol. 16, 634–642
14. Routledge, E. J., White, R., Parker, M. G., and Sumpter, J. P. (2000) J. Biol. Chem. 275, 35986–35993
15. Paffi, M. W. (2001) Nuclear Acids Res. 29, e45
16. Weismann, A. S., Mitchell, D. M., Sanjabi, S., Bradley, M. N., Hoffmann, A., Liou, H. C., and Smale, S. T. (2001) Nat. Immunol. 2, 51–57
17. Fujita, N., Jaye, D. L., Kajita, M., Geigerman, C., Moreno, C. S., and Wade, M. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 113–117
18. Smale, S. T., and DallaBorgo, M. (1998) Cell 97, 103–113
19. Burke, T. W., and J. T. Kadonaga (1996) Gene (Amst.) 16, 711–724
20. Philippis, S., and Suske, G. (1999) Nucleic Acids Res. 27, 2991–3000
21. Bouwman, P., and Philippis, S. (2002) Mol. Cell. Endocrinol. 195, 27–38
22. Macleod, D., Charlton, J., Mullins, J., and Bird, A. P. (1994) Genes Dev. 8, 2282–2292
23. Zhu, W. G., Srinivasan, K., Dai, Z., Duan, W., Druhan, L. J., Ding, H., Yee, L., Villalona-Calero, M. A., Plass, C., and Otteson, G. A. (2003) Mol. Cell. Biol. 23, 4656–4665
24. Kaczynski, J. Z., Zhang, J. S., Ellenrieder, V., Conley, A. D., Kester, H. T., van den Brink, B. B., and Rurutia, R. (2001) J. Biol. Chem. 276, 36749–36756
25. Kwon, H. S., Kim, M. S., Edenberg, H. J., and Hur, M. W. (1999) J. Biol. Chem. 274, 20–29
26. Gregory, R. C., Taxman, D. J., Sebaaaseye, D., Kensing, M. H., Bieker, J. J., and Wojcikowski, D. M. (1996) Blood 87, 1793–1801
27. Karlseder, J., Rotheneder, H., and Wintersberger, E. (1994) Mol. Cell. Biol. 16, 1659–1667
28. Lee, S. J., Galvin, K. M., and Shi, Y. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6541–6149
29. Lin, S. Y., Black, A. R., Kostic, D., Pajovic, S., Hoover, C. N., and Azizkhan, J. C. (1996) Mol. Cell. Biol. 16, 1668–1675
30. Datta, P. K., Raychaudhuri, P., and Bagchi, S. (1995) Mol. Cell. Biol. 15, 5444–5452
31. Vayou, S., Chin, K. V., and Chang, K. S. (1998) Mol. Cell. Biol. 18, 7147–7156
32. Lee, D. K., Subh, D., Edenberg, H. J., and Hur, M. W. (2002) J. Biol. Chem. 277, 26761–26768
33. Suzuki, T., Muto, S., Miyamoto, S., Aizawa, K., Horiuchi, M., and Nagai, R. (2003) J. Biol. Chem. 278, 26764–26768
34. Johnson-Pais, T., Degnin, C., and Thayer, M. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 2211–2216
35. Doetzhofer, A., Rotheneder, H., Lagger, G., Koranda, M., Kurtev, V., Brossch, M., Maser, A., Wintersberger, E., and Seiser, C. (1999) Mol. Cell. Biol. 19, 5504–5511
36. Lai, A., Lee, J. M., Yang, W. M., DeCaprio, J. A., Kaelin, Jr., W. G., Seto, E., and Branton, P. E. (1999) Mol. Cell. Biol. 19, 6632–6641
37. Sun, H., and Taneya, R. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4058–4063
38. Fernandez, I., Bastien, Y., Wai, T., Nygard, K., Lin, R., Cornier, O., Lee, H. S., Eng, F., Bertos, N. R., Pelletier, N., Mader, S., Han, V. K., Yang, X. J., and White, J. H. (2003) Mol. Cell. 11, 139–150