Epigenetic Control of the Immune Escape Mechanisms in Malignant Carcinomas

A. Francesca Setiadi,1,2,3 Muriel D. David,1 Robyn P. Seipp,1,2,3 Jennifer A. Hartikainen,1,2,5 Rayshad Gopaul,2 and Wilfred A. Jefferies1,2,3,4,5*

Biomedical Research Centre, Vancouver, British Columbia V6T 1Z3, Canada; Michael Smith Laboratories, Vancouver, British Columbia V6T 1Z4, Canada; Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada; Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada; and Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

Received 23 August 2007/Accepted 30 August 2007

Downregulation of the transporter associated with antigen processing 1 (TAP-1) has been observed in many tumors and is closely associated with tumor immunoavasion mechanisms, growth, and metastatic ability. The molecular mechanisms underlying the relatively low level of transcription of the tap-1 gene in cancer cells are largely unexplained. In this study, we tested the hypothesis that epigenetic regulation plays a fundamental role in controlling tumor antigen processing and immune escape mechanisms. We found that the lack of TAP-1 transcription in TAP-deficient cells correlates with low levels of recruitment of the histone acetyltransferase, CBP, to the TAP-1 promoter. This results in lower levels of histone H3 acetylation at the TAP-1 promoter, leading to a decrease in accessibility of the RNA polymerase II complex to the TAP-1 promoter. These observations suggest that CBP-mediated histone H3 acetylation normally relaxes the chromatin structure around the TAP-1 promoter region, allowing transcription. In addition, we found a hitherto-unknown mechanism wherein interferon gamma up-regulates TAP-1 expression by increasing histone H3 acetylation at the TAP-1 promoter locus. These findings lie at the heart of understanding immune escape mechanisms in tumors and suggest that the reversal of epigenetic codes may provide novel immunotherapeutic paradigms for intervention in cancer.

The current paradigm suggests that the emergence of tumors is limited by a robust adaptive immune response that recognizes aberrant expression of tumor-associated antigens. This mechanism of immune surveillance is thought to work efficiently until tumor cells undergo chromosomal alterations that result in phenotypic conversion to a form that is no longer recognizable by the immune system. This conversion closely parallels the emergence of metastatic forms of the tumor cells that in turn gain a growth advantage over nonmetastatic forms that remain hampered by the fidelity of the immune system.

Several immune escape mechanisms that allow the metastatic tumor to go undetected have been observed; however, most tumors down-regulate a cassette of genes involved in antigen processing and presentation (7, 27, 28). These genes include those for beta-2 microglobulin, the transporters associated with antigen processing 1 and 2 (TAP-1 and -2), tapasin, the low-molecular-weight proteins LMP-2 and -7, and the proteasome activator PA28 (7, 16, 29). TAP-1 down-regulation has specifically been attributed to tumor growth and metastatic ability and is used as a predictor of rapid tumor progression and poor survival rates in humans (1, 7, 11, 12, 16, 26–28). Furthermore, this antigen presentation deficiency can be temporarily reversed in vitro by treatment of the tumor cells with gamma interferon (IFN-γ) and can also be genetically complemented in vivo by the sole restoration of TAP-1 expression (1, 7). Therefore, the presence of TAP-1 improves the ability of the host to mount a therapeutic and protective antitumor immune response. This finding is encouraging for the development of therapeutic approaches that can restore TAP deficiency in cancer cells and thus result in restoration of immune recognition of tumors. However, the actual defects that lead to TAP downregulation and immune escape mechanisms remain undescribed.

Our previous study concluded that TAP deficiency in metastatic carcinomas is not caused by mutations within the TAP-1 promoter. We found that metastatic carcinomas lack positive trans-acting factors that regulate TAP-1 transcription (30). At the initiation of the present study, we were intrigued by our observation that transient transfection of episomal copies of a reporter gene driven by the TAP-1 promoter led in TAP-deficient carcinomas to expression levels of the reporter gene similar to those in TAP-expressing cells. In contrast, stable transfection and genomic integration of the same plasmid revealed the differential activity of the TAP-1 promoter between TAP-deficient and TAP-expressing cells. This led us to investigate whether epigenetic mechanisms play a role in the regulation of antigen processing in tumors and whether the transcriptional activators that are deficient or nonfunctional in malignant cells are those with intrinsic histone acetyltransferase (HAT) activity. Our present study provides fundamental insights into the molecular basis of tumor immune escape and metastatic diseases, which may help in revising immunotherapeutic methodologies for eradicating cancers.

**MATERIALS AND METHODS**

**Cell lines and reagents.** The TC-1 cell line was developed by transformation of murine primary lung cells with human papillomavirus type 16 (HPV16) E6 and E7 oncogenes and activated H-ras (19). TC-1 cells display high levels of TAP-1...
and major histocompatibility complex (MHC) class I, TC1-/D11 (≈ D11) and TC1-/A9 (≈ A9) are metastatic clones that were derived from the TC-1 tumor and that display spontaneous downregulation of MHC class I expression (31). These cell lines were cultured in the presence of 0.4 mg/ml G418. We also studied another model consisting of a murine primary prostate cancer cell line, PA, and its metastatic, TAP- and MHC class I-deficient derivative, LMD, both derived from a 129/Sv mouse (17). The TAP-deficient CMT-64 cell line was established from a spontaneous lung carcinoma in a C57BL/6 mouse (6). Heat-inactivated fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, and 10 mM HEPES. When indicated, cells were treated with 50 ng/ml IFN-γ.

Protein A Sepharose CL-4B beads (Amersham Biosciences, Uppsala, Sweden) and major histocompatibility complex (MHC) class I, TC1-/D11 (≈ D11) and TC1-/A9 (≈ A9) are metastatic clones that were derived from the TC-1 tumor and that display spontaneous downregulation of MHC class I expression (31). These cell lines were cultured in the presence of 0.4 mg/ml G418. We also studied another model consisting of a murine primary prostate cancer cell line, PA, and its metastatic, TAP- and MHC class I-deficient derivative, LMD, both derived from a 129/Sv mouse (17). The TAP-deficient CMT-64 cell line was established from a spontaneous lung carcinoma in a C57BL/6 mouse (6).

**Flow cytometry.** Flow cytometric analysis of H-2Kα expression was performed using phycocerythrin-conjugated anti-Kβ mouse monoclonal antibody (BD Pharmingen, San Diego, CA) and a FACScan cytometer (Becton Dickinson, Mountain View, CA).

**Chromatin immunoprecipitation assays.** Chromatin immunoprecipitation experiments using 7 × 10⁶ cells per sample were done as previously described (9). Protein A Sepharose CL-4B beads (Amersham Biosciences, Uppsala, Sweden) and 5 μg of anti-RNA polymerase II (Pol II) (N-20, sc-899; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-acetyl-histone H3 (Upstate Biotechnology, Inc., Lake Placid, NY), or anti-CBP (A-22, sc-369; Santa Cruz Biotechnology) polyclonal antibody were used for immunoprecipitation. Mock coprecipitations without antibody were performed for each sample, in parallel with the coimmunoprecipitations, to detect any nonspecific coprecipitation of genomic DNA with protein A-Sepharose beads. Levels of endogenous TAP-1 promoter communoprecipitating with the antibody from each sample were quantified by real-time PCR using primers specific for the TAP-1 promoter. Primers specific to the 3′ end of the TAP-1 promoter region were used for templates immunoprecipitated using anti-RNA Pol II or anti-acetyl-histone H3 antibody, while 5′-end-specific primers were used for templates immunoprecipitated using anti-CBP antibody (Table 1). Relative RNA Pol II or acetyl-histone H3 levels were determined as the ratio of copy numbers of the eluted TAP-1 promoter to copy numbers of the corresponding inputs. Serial dilutions of plasmid containing the murine TAP-1 promoter were amplified following the same protocol to generate a standard curve.

**Plasmid construction.** The plasmid containing an enhanced green fluorescent protein (EGFP) coding region driven by the TAP-1 promoter (pTAP1-EGFP) was described previously (30). A similar construct containing a luciferase (luc) gene driven by the TAP-1 promoter region (pTAP1-Luc) was created by inserting the TAP-1 promoter between the Sacl and BglII sites of the plgLA14[luc2/Hygro] vector (Promega, Madison, WI). 5′-End truncations of the TAP-1 promoter region were also cloned into the plgLA14[luc2/Hygro] vector. The position of the ATG codon of the tap-1 gene was arbitrarily numbered +1, and the truncated promoters were named according to the starting base position of forward primers with respect to the ATG codon (−427, −401, and −150). Primers used for PCR amplifications of the full TAP-1 promoter and its truncations are listed in Table 1.

**Transfection and selection.** TC-1, D11, A9, PA, and LMD cells were transfected with the pTAP1-Luc constructs or the promoterless plgLA14[luc2/Hygro] vector using ExGen 500 in vitro transfection reagent (Fermentas). Transient transfectants were analyzed between 12 and 72 h after cotransfection of the pTAP1-Luc construct or the promoterless vector with the pRL-TK plasmid (Promega) in a 10:1 ratio. To obtain stable transfectants, the transfected cells were selected for 3 weeks in the presence of 200 to 550 ng/ml hygromycin B (Sigma).

**Luciferase assays.** Luciferase activity in transient transfectants was assessed by using a dual-luciferase assay (Promega) 12 to 72 h after transfection. Luciferase activity in stable transfectants (3 to 4 weeks posttransfection) was assessed by using the Bright-Glo luciferase assay (Promega) using 10,000 cells per sample. The luciferase values obtained were normalized to the copy number of plasmids integrated into the genome of each stable transfectant. Copy numbers of the pTAP1-Luc construct integrated into the genome of the stable transfectants were quantified by real-time PCR using a forward primer specific for the TAP-1 promoter and a reverse primer specific for the luc2 gene.

### Table 1. Primers used for RT-PCR and real time PCR analysis

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Primer sequence (5′→3′)</th>
<th>Tm (°C)</th>
<th>No. of bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAP-1</td>
<td>F: TGGCTCTTGTTGGCACCCTCTAAA</td>
<td>64.0</td>
<td>775</td>
</tr>
<tr>
<td></td>
<td>R: TCAGTCTGCAAGCGGCCGACAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>F: AATGGATCCAGATATCGCTC</td>
<td>54.0</td>
<td>713</td>
</tr>
<tr>
<td></td>
<td>R: TTCTCCAGGAGAGAACGAGAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full TAP-1 promoter</td>
<td>F: cgagagctcGCTCGTCTTCCATCA</td>
<td>60.0</td>
<td>557</td>
</tr>
<tr>
<td></td>
<td>R: gaagatctGAGGTACGTCGACTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−427 truncated TAP-1 promoter</td>
<td>F: cgagagctcATCTGCCCAGAGACAGGTGA</td>
<td>55.0</td>
<td>427</td>
</tr>
<tr>
<td></td>
<td>R: gaagatctGAGGTACGTCGACTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−401 truncated TAP-1 promoter</td>
<td>F: cgagagctcAGGGTCTCCTGCCTCAATTC</td>
<td>55.0</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td>R: gaagatctGAGGTACGTCGACTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−150 truncated TAP-1 promoter</td>
<td>F: cgagagctcTCTTACTGACAGCTCAGCAGTCTC</td>
<td>60.0</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>R: gaagatctGAGGTACGTCGACTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAP-1 promoter (5′-end)</td>
<td>F: GCCTGCTCTTTCCAAATCA</td>
<td>60.0</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>R: GGATGCGGAAAATTTACCGCAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAP-1 promoter (3′-end)</td>
<td>F: TTCTTCCTTAAACGCGCACA</td>
<td>61.0</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>R: CGAGGTCAGCTGTCGAGTCTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pTAP1-Luc copy number</td>
<td>F (TAP-1 promoter): TTCTTCCTTAAACGCGCACA</td>
<td>61.0</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>R (luc2): AGTGGGTAGAATGGCGCTG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* F, forward primer; *R*, reverse primer. Lowercase letters are additional nucleotide sequences containing restriction enzyme sites (underlined).

*b* Length of the PCR amplification product.
Western blots. Fifty micrograms of protein per sample was separated with 6% (CBP) or 15% (β-actin) sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). Blots were blocked with 5% skim milk in phosphate-buffered saline and incubated with the anti-CBP (A-22, sc-369; Santa Cruz) rabbit polyclonal antibody. Secondary antibody was a horseradish peroxidase-conjugated goat antirabbit polyclonal antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). For the loading controls, anti-β-actin mouse monoclonal antibody (Sigma) was used, followed by horseradish peroxidase-conjugated goat antimouse secondary antibody (Pierce, Rockford, IL). Blots were visualized using Lumi-light ECL reagents (Pierce).
RESULTS

Chromatin remodeling regulates TAP-1 transcription. In order to investigate transcriptional regulation of TAP-1, we generated a luciferase reporter construct by cloning the mouse TAP-1 promoter region upstream of the luciferase gene in the pGL4.14[luc2/Hygro] vector (pTAP1-Luc) and transfected this construct into the TAP-expressing (Ltk and RMA) and TAP-deficient (LMD, CMT.64, and B16F10) cells that we used in our previous study (30). Consistent with our previous observations with the TAP-1 promoter–EGFP reporter construct (30), we found that the pattern of expression of luciferase in these stable transfectants correlated with the pattern of endogenous TAP-1 mRNA expression (30) (Fig. 1A, upper panels). However, when the TAP-expressing and TAP-deficient cells were transiently transfected with the same construct, the pattern of luciferase expression no longer correlated with endogenous TAP-1 mRNA levels (Fig. 1A, upper panels).

It was conceivable that this lack of correlation had resulted, at least in part, from the fact that the cell lines used were derived from distinct types of carcinomas and therefore were unrelated to each other. To avoid this problem, we used two other models: the PA/LMD group and the TC-1/D11/A9 group. Each model consists of one primary, TAP-expressing cancer cell line (TC-1 or PA) and at least one metastatic, TAP-deficient cancer cell line (D11, A9, or LMD) that we used in our previous study (30). Consistent with our previous observations with the TAP-1 promoter–EGFP reporter construct (30), we found that the pattern of expression of luciferase in these stable transfectants correlated with the pattern of endogenous TAP-1 mRNA expression (30) (Fig. 1A, upper panels). However, when the TAP-expressing and TAP-deficient cells were transiently transfected with the same construct, the pattern of luciferase expression no longer correlated with endogenous TAP-1 mRNA levels (Fig. 1A, upper panels).

Among all the tumor cell lines tested, the only exception to this model was the LMD cells. Like all the other TAP-deficient cell lines, the LMD cells expressed a very low level of TAP-1 and MHC class I. Similarly, in the HPV-positive carcinoma model, TC-1, D11, and A9 cell lines that expressed high, moderate, and low levels of TAP-1, respectively, also expressed the same pattern of surface MHC class I. These TAP-expressing and TAP-deficient cells were then transfected with the pTAP1-Luc construct. With the TC-1/D11/A9 model, we found that, again, the pattern of the relative levels of luciferase activity correlated with the pattern of endogenous TAP-1 mRNA expression only in stable population of transfectants (Fig. 1A, middle panels). Thus, in transient transfecnts, levels of luciferase activity were similar in TAP-expressing and TAP-deficient cells (Fig. 1A, middle panels). In all experiments, we verified as a control that the levels of luciferase activity yielded by the promoterless pGL4.14[luc2/Hygro] vector were similar in all the transfected cell lines and were negligible compared to those yielded by the pTAP1-Luc construct (Fig. 1A). Taken together, the results of the transfection experiments using the TAP-expressing cells (Ltk and RMA), the TAP-deficient cells (LMD, CMT.64, and B16F10), and the TC-1/D11/A9 model suggest that integration of the reporter construct into the chromatin is required to reveal the difference in TAP-1-promoter activity between TAP-expressing and TAP-deficient cells. Moreover, we found that binding of RNA Pol II to the endogenous TAP-1 promoter was lower in TAP-deficient cells than in TAP-expressing cells (Fig. 2A). One possible explanation to account for this phenomenon is that in TAP-deficient cells, the chromatin structure is forming a physical barrier that reduces the access of the RNA Pol II complex to the TAP-1 promoter.

FIG. 2. RNA Pol II and acetyl-histone H3 binding to the TAP-1 promoter is low in TAP-deficient carcinomas. The level of RNA Pol II (A) or acetyl-histone H3 (B) in the TAP-1 promoter of each cell line was assessed by chromatin immunoprecipitation using anti-RNA Pol II or anti-acetyl-histone H3 antibody, respectively. The eluted DNA fragments were purified and used as templates for real-time PCR analysis using primers specific for the 3′ end of the TAP-1 promoter. To determine the relative levels of binding of RNA Pol II or acetyl-histone H3 to the TAP-1 promoter, the copy numbers of the eluted TAP-1 promoter were normalized against the copy numbers of the corresponding inputs. The results were then expressed as ratios of the values obtained with the TAP-expressing cell line to the values obtained with the TAP-deficient cell line in the same experimental model. Note that histone H3 acetylation was absent in the TAP-1 promoter of CMT.64 cells. Columns, average for three to six independent experiments; bars, standard errors of the means. *, $P < 0.05$ compared with results for cells that expressed higher TAP-1 and MHC class I levels in the same experimental model (Student’s $t$ test).
cells, LMD cells displayed a relatively low level of binding of RNA Pol II to the endogenous TAP-1 promoter (Fig. 2A). However, both the stable and the transient transfections of these cells with the pTAP1-Luc construct yielded relatively low levels of luciferase activity that correlated with the deficiency in endogenous TAP-1 mRNA expression (Fig. 1A, lower panels). Thus, LMD cells, the only TAP-deficient cells that displayed low levels of luciferase activity when transiently transfected, are likely to have other defects that impair the activity of the TAP-1 promoter in addition to those related to chromatin remodeling.

**Histone H3 acetylation within the TAP-1 promoter is low in MHC class I-deficient carcinomas.** A well-known epigenetic mechanism that regulates gene expression is the acetylation of histone H3 within a gene locus (2, 4, 21), which promotes relaxation of the nucleosome structure and allows the transcriptional machinery to access the promoter (4, 5). Therefore, we tested the hypothesis that differential TAP-1 promoter activity between TAP-expressing and TAP-deficient cells results from distinct levels of histone H3 acetylation within the TAP-1 promoter.

In the HPV-positive carcinoma model, we found that histone H3 acetylation within the TAP-1 promoter was impaired in A9 cells (Fig. 2B), which express the lowest level of TAP-1. D11 cells, which express intermediate levels of TAP-1 compared to those of TC-1 and A9 cells, also had intermediate levels of acetyl-histone H3 within the TAP-1 promoter (Fig. 2B). Similarly, with the prostate carcinoma model, the metastatic, TAP-deficient LMD cells had less acetyl-histone H3 within the TAP-1 promoter than was found in the nonmetastatic, TAP-positive PA cells. We also assessed the levels of acetyl-histone H3 within the TAP-1 promoter in several other TAP-expressing (Ltk and RMA) and TAP-deficient (CMT.64 and B16F10) cell lines (Fig. 2B). Again, lower levels of acetyl-
histone H3 were found in the TAP-deficient carcinomas; in fact, acetyl-histone H3 was undetectable in the highly metastatic CMT.64 cells. Taken together, these results demonstrate a clear correlation between the levels of endogenous TAP-1 mRNA and histone H3 acetylation within the TAP-1 promoter.

**Identification of the region in the TAP-1 promoter responsible for the differential activity between TAP-expressing and TAP-deficient cells.** In order to determine the critical region of the TAP-1 promoter that is responsible for the differential promoter activity in TAP-expressing and TAP-deficient cells, several constructs were made by cloning 5′-end truncations of the TAP-1 promoter into the pGL4.14[luc2/Hygro] vector (constructs −427, −401, and −150 in Fig. 3A). We found that progressive truncations of the 5′ end of the TAP-1 promoter resulted in a gradual decrease in the promoter activity (as measured by luciferase expression) in the TAP-expressing, TC-1 and PA cells (Fig. 3B). This decrease in promoter activity resulting from the truncations indicates that the deleted regions normally participate in TAP-1 promoter activity. Interestingly, the truncations up to −401 did not affect the activity of the TAP-1 promoter in the TAP-deficient A9 and LMD cells. This indicates that in contrast to what we observed in the TAP-expressing TC1 and PA cells, the −567 to −401 region of the TAP-1 promoter does not participate in the control of TAP-1 promoter activity in TAP-deficient cells. Finally, it is important to note that the −401 construct yielded similar promoter activity in both TAP-expressing and TAP-deficient cells (Fig. 3B). Taken together, these observations indicate that all the cis-acting elements responsible for the differential TAP-1 promoter activity in TAP-expressing versus TAP-deficient cells are located in the region encompassing nucleotides −567 to −401.

A more detailed analysis revealed that the region encompassing nucleotides −427 to −401 was sufficient to confer the differential TAP-1 promoter activity observed between TAP-expressing and TAP-deficient cells (Fig. 3B). Analysis of putative transcription factor binding sites, using Tsitescan software (www.ifi.org), suggested the presence of a CREB binding site within this region (Fig. 3A).

**Binding of CBP to TAP-1 promoter is impaired in metastatic carcinomas.** The existence of a putative CREB binding site in the region responsible for the differential activity of the TAP-1 promoter between TAP-expressing and TAP-deficient cells is particularly interesting, since the CBP is one of the well-known transcriptional coactivators that possess intrinsic HAT activity (8, 13, 18). In addition, CBP is known to acetylate histone H3 (3), and the HAT activity and recruitment of CBP to promoters can be stimulated by various transcription factors (18), including SP-1 and AP-1, whose binding sites are also found in the TAP-1 promoter (Fig. 3A). These notions are consistent with our hypotheses that the differential TAP-1 promoter activity results from differences in chromatin structure at the tap-1 locus and that the trans-acting factors deficient or nonfunctional in TAP-deficient carcinomas can be those with the ability to control chromatin structures. Therefore, we assessed whether CBP plays a role in the differential TAP-1 promoter activity between TAP-expressing and TAP-deficient cells.

Western blot analysis showed that CBP is not lacking or truncated in the TAP-deficient A9 and LMD cells derived from metastatic carcinomas (Fig. 4A). However, chromatin immunoprecipitation analysis showed that binding of CBP to the TAP-1 promoter was significantly lower in the TAP-deficient A9 and LMD cells than in the TAP-expressing TC-1 and PA cells (Fig. 4B). These results suggest that in TAP-deficient cells, the lack of HAT activity normally exerted by CBP in the TAP-1 promoter is likely to play a role in dictating the inaccessibility of the promoter to the RNA Pol II complex and in the subsequent impairment of TAP-1 transcription.

**IFN-γ treatment increases the level of CBP, acetyl-histone H3, and RNA Pol II recruitment to the TAP-1 promoter in TAP-deficient metastatic carcinomas.** Since the lack of CBP-mediated acetylation of histone H3 at the TAP-1 promoter appeared to contribute to the TAP-1 deficiency observed in metastatic carcinomas, we tested whether IFN-γ, a well-known inducer of TAP-1 expression (17, 23, 24, 30), restores
TAP-1 expression by increasing histone H3 acetylation at this locus. We found that, indeed, treatment of the TAP-deficient A9 and LMD cells with IFN-γ increased the levels of CBP, acetyl-histone H3, and RNA Pol II recruitment to the TAP-1 promoter (Fig. 5). Strikingly, in the presence of IFN-γ, the TAP-expressing TC-1 cells and the TAP-deficient A9 cells were no longer distinguishable with respect to their level of CBP, acetyl-histone H3, and RNA Pol II recruitment to the TAP-1 promoter. This was also true for the PA/LMD model, with the exception of RNA Pol II recruitment, which despite the treatment with IFN-γ was still somewhat lower in the TAP-deficient LMD cells. Based on these results, we propose that a possible mechanism of TAP-1 induction by IFN-γ is via the restoration of CBP binding to the TAP-1 promoter, which in turn promotes histone H3 acetylation in the TAP-1 promoter region and the subsequent relaxation of the surrounding chromatin. This remodeling of the chromatin would in turn increase the accessibility of the RNA Pol II complex to the TAP-1 promoter and promote transcription of this gene.

**DISCUSSION**

The regulation of chromatin structure plays an important role in controlling gene expression. Indeed, a compact nucleosome structure around a promoter region can act as a physical barrier that prevents the binding of transcriptional activators to the promoter and consequently halts the transcription process (10). Active transcription has been associated with high acetylation levels of core histones in the chromatin (2, 4, 5, 18, 32), particularly in the proximal region of acetylation-sensitive promoters (10, 32). Although histone H3 is not the only core histone whose modification has been shown to influence gene expression (2, 14), the correlation between the acetylation of histone H3 and activation of several genes has been widely studied and is now well established (2, 4, 14, 21).
Our observations that silencing of the TAP-1 promoter reporter construct in TAP-deficient cells is revealed exclusively after the construct integrates into the genome (Fig. 1) and that levels of RNA Pol II bound to the TAP-1 promoter are relatively low in metastatic, TAP-deficient cells (Fig. 2A) suggest that access of the RNA Pol II complex to the TAP-1 promoter is limited in TAP-deficient cells. Thus, it is likely that TAP deficiency in metastatic cancer cells results, at least in part, from a repressive state of the chromatin. Consistent with this notion, we found that the region in the TAP-1 promoter that is responsible for its differential activity between the TAP-expressing and TAP-deficient carcinoma cells encompasses a binding site for CREB (Fig. 3A), a protein known to interact with the histone acetyltransferase CBP (8, 13). CBP is known to acetylate histones around promoter regions, resulting in increased accessibility of the promoters to essential transcriptional regulators (13). We observed that the recruitment of CBP to the TAP-1 promoter and the acetylation level of histone H3 within the TAP-1 promoter were lower in TAP-deficient cells than in TAP-expressing cells (Fig. 2B and 4B). Our findings suggest that the level of recruitment of CBP to the TAP-1 promoter, by controlling the level of histone H3 acetylation at the TAP-1 promoter locus, plays a critical role in the regulation of TAP-1 transcription. Down-modulation of the recruitment of CBP to the TAP-1 promoter in metastatic cancer cells results in a decreased level of histone H3 acetylation at the TAP-1 promoter, likely leading to TAP deficiency by promoting a compact nucleosome structure that disables the recruitment of RNA Pol II to the TAP-1 promoter. In our previous study, we proposed that the lack of expression or activity of trans-acting factors normally recruited to the TAP-1 promoter is one of the mechanisms that contribute to TAP deficiency in malignant cells (30). CBP is likely to be one of these factors. The exact mechanisms responsible for the decreased recruitment of CBP to the TAP-1 promoter remain to be identified.

Although the decrease in histone H3 acetylation appears to be a hallmark of metastatic cells, it is not likely to be the sole mechanism involved in TAP-1 deficiency. The PA/LMD model presented in this study is a clear illustration of the complexity of the molecular mechanisms leading to TAP deficiency: in addition to the decrease in histone H3 acetylation within the TAP-1 promoter, these cells exhibit an impaired activity of the TAP-1 promoter that is detectable even in the context of transient transfection of episomal copies of a reporter gene driven by the TAP-1 promoter. Moreover, we previously demonstrated that TAP deficiency results not only from the relatively low activity of the TAP-1 promoter but also from a decrease in the stability of TAP-1 mRNA (30). Thus, several molecular mechanisms concur to decrease TAP-1 expression in metastatic cells.

IFN-γ is known to be a potent inducer of TAP-1 and surface MHC class I expression in cancer cells (17, 23, 24, 30); however, little is known about molecular mechanisms that lead to induction of TAP-1 by IFN-γ. We found that treatment of TAP-deficient cells with IFN-γ increased the binding of CBP to the TAP-1 promoter, up to levels similar to those observed in TAP-expressing cells (Fig. 5). This was accompanied by an increase in levels of histone H3 acetylation that correlated with an increase in RNA Pol II recruitment to the TAP-1 promoter and active transcription of the tap-1 gene. Thus, our results provide a novel mechanism by which IFN increases TAP-1 expression.

Deregulation of genes involved in the modulation of chromatin structure has been closely linked to uncontrolled cell growth and development of tumors (15, 20, 22). Our study provides support for the notion that TAP-deficient carcinomas lack trans-acting factors that would normally enable relaxation of the chromatin structure to allow access of general transcription factors and RNA Pol II to the promoter of the tap-1 gene. These data provide the first insights into the epigenetic mechanisms responsible for the immune escape of cancer cells. Further research that aims to identify additional chromatin-remodeling factors that play roles in tumor antigen processing deficiencies will be essential for the development of novel therapeutic approaches against MHC class I-deficient cancers. In conclusion, the reversal of epigenetic codes may provide new targets and modalities for therapeutic intervention in cancer.

ACKNOWLEDGMENTS

We thank T. C. Wu for providing the TC-1 cell line, M. Smahel for providing the TC-1/D11 and TC-1/A9 cell lines, T. C. Thompson for providing the LMD cell line, Andy Johnson for assistance with the flow cytometry analysis, Karen Chang and Shaun Sanders for technical assistance, and Karl-Erik Hellstrom, John Schrader, Tim Vitalis, and Kyung Bok Choi for their helpful suggestions and comments.

This work was supported by grants from Canadian Institutes of Health Research, National Cancer Institute of Canada and the Prostate Cancer Research Foundation of Canada. A.F.S was supported by the William and Dorothy Gilbert Graduate Scholarship in Biomedical Sciences. R.P.S. was supported by the Natural Sciences and Engineering Research Council of Canada and the Michael Smith Foundation for Health Research.

REFERENCES


22. Reference deleted.


