The Sp Family of Transcription Factors in the Regulation of the Human and Mouse MUC2 Gene Promoters

Fauzia Aslam, Lisa Palumbo, Leonard H. Augenlicht, and Anna Velcich

Department of Oncology, Albert Einstein Cancer Center/Montefiore Medical Center, Bronx, New York 10467

ABSTRACT

Modulation of mucin gene expression is an important component both in the early steps of colon cancer development and in later tumor progression. Previous work from our laboratory and others has suggested that the Sp family of transcription factors may play an important role in the regulation of the human MUC2 gene. To determine whether this was an essential element, we extended our work to the cloning and analysis of 3.5 kb of the 5'-flanking region of the mouse Muc2 (mMuc2) gene. Comparative analysis between the mouse and human MUC2 promoter regions has identified a strong sequence homology between the mouse and human genes, including the presence of GC-rich boxes, the location and composition of which are maintained in the mouse and human genes. We show that these GC boxes are binding sites for Sp-family transcription factors and are functionally important since mithramycin, an inhibitor of Sp1/Sp3 binding, blocks mMUC2 gene expression in HT29 cells. Furthermore, by a combination of gel shift analysis and site-directed mutagenesis, we have identified the relative contribution of individual GC boxes, and of the factors they bind, to the regulation of the mouse Muc2 promoter, which appears to be different in the mouse and human genes. Finally, we demonstrate by overexpressing Sp1 and Sp3 that the functional difference between the proximal promoter region of the MUC2 gene in the two species is not attributable to differential ability of this region to bind members of the Sp family of transcription factors, but rather to the different anatomy of the individual GC boxes in the mouse and human proximal promoters.

INTRODUCTION

Mucins are highly glycosylated molecules and comprise the major component of mucus, the viscoelastic substance that protects and lubricates the epithelia mucosa, including the gastrointestinal mucosa. The major form of secreted gastrointestinal mucin (1) is encoded by the MUC2 gene, which is expressed specifically in the goblet cells of the intestine. Thus, MUC2 represents a fundamental marker for elucidating mechanisms that regulate differentiation of the secretory cell lineage.

Modulation of mucin gene expression may be an important component both in the early steps of colon cancer development and in later tumor progression. For example, it has been reported that in aberrant crypt foci, morphological alterations in the flat mucosa that are considered early putative neoplastic lesions (2, 3), there is a lack of representation of goblet cells and therefore of the mucus they secrete (4). These abnormalities are recapitulated in the mouse Apc1638 model (5), which has a genetically inactivated Apc allele and spontaneously develops intestinal tumors (6). Qualitative and/or quantitative alterations in mucin glycosylation characterize later stages of colon tumor progression, and the recent availability of probes for the genes that encode mucin peptides has made it possible to study alterations of apomucin expression; both levels and pattern of expression of mucin genes are altered in adenocarcinoma compared with normal tissues, and these changes correlate with the cancer phenotype (7).

Extensive work from our lab, as well as others, has focused on the regulation of the human MUC2 gene (8–13). Sequence analysis of the human MUC2 promoter revealed the presence of several GC boxes, putative binding sites for the Sp family of transcription factors, which have been suggested to play a role in the regulation of the MUC2 gene (11, 12). Accordingly, we now report that mithramycin, an inhibitor of Sp1 binding, blocked the expression of MUC2 in the HT29 adenocarcinoma cell line. To prove the role of the Sp family factors in the regulation of MUC2, we extended our work to the cloning, structural analysis, and functional analysis of the promoter of the mouse Muc2 gene, which has not been reported. In our structural characterization of the mouse Muc2 promoter, we have identified a strong sequence homology between the mouse and human genes in the region immediately upstream of the transcription initiation site. This conservation may suggest functional relevance, also indicated by a similar pattern of expression that is shared by the mouse and human MUC2 genes in the intestine of the two species (14). The mouse proximal promoter region, similar to the corresponding region in the human MUC2 promoter, contains several GC boxes that we show are binding sites for the Sp family of transcription factors, and we show that there are distinct binding patterns of Sp1 and Sp3 to the mouse and human proximal promoters. However, our data demonstrate that the functional difference between the proximal promoter region in the mouse and human MUC2 genes is not attributable to a differential ability of this region to bind members of the Sp family of transcription factors, but rather to the different anatomy of the individual GC boxes in the mouse and human promoters.

MATERIALS AND METHODS

Genomic Cloning of the Mouse Muc2 Gene, and Muc2/luciferase Plasmid Construction. A mouse Charon 35, 129/0la phage genomic library was screened with a PCR-generated 1.4 kb probe amplified from mouse DNA using the following primers: forward 5’-GAAACCGCTTCTGAGGTAC-3’ and reverse 5’-AAATGCCGTCAGCTTAAG-3’ of the published rat Muc2 cDNA sequence (15). Two overlapping clones that spanned approximately 15 kb were isolated, and each was subcloned into BlueScript SK+ vector (Stratagene); a restriction map was derived by analysis of partial restriction enzyme digests. For sequence analysis, the BlueScript double-stranded DNA templates were sequenced with T3, T7, and internal primers using the Sequenase kit, version 2 (United States Biochemical). Nucleic acid sequence data analysis was performed with Wisconsin Sequence Analysis Package GCG software (Genetics Computer Group, Madison, WI).

To generate the mMuc2/luciferase reporter vectors, different portions of the 3.5 kb sequence upstream of the translational start site, as shown in Fig. 7, were cloned into the pGL2basic vector (Promega), which contain no regulatory elements. The 3.5 kb SacI-NcoI most 5’ fragment of the mMuc2 promoter (Fig. 2A) was blunt ended at the NcoI site, corresponding to the AUG initiation codon, HindIII linkers were added, and the fragment was then cloned into the HindIII-SacI site of pGL2 basic generating the m-3.5 kb construct. The m-3.5 kb plasmid was partially digested with DraI, blunt ended, and the 1.2 kb DraI/HindIII fragment was cloned into the Smal/HindIII sites of the pGL2 basic...
nuclear proteins were incubated with 10 μl of mithramycin (Sigma Chemical Co.) before being used in binding reactions. The mouse mutant Sp1/AP2 plasmids were constructed with the MORPH site-directed mutagenesis kit (5 prime-3 prime, Inc.) using an oligonucleotide, spanning from nt −94 to −59, with a mutated Sp1/AP2 site (wt 5’CCGCCTCTCGG3’; mutant oligo 5’CCatCactGGA3’). 

Gel Mobility Shift Assay. Nuclear extracts were prepared from HT29 cells using the NP40 detergent lysis buffer and 0.4 μM NaCl extraction buffer method, as described by Schreiber et al. (16). Protein concentration was determined by the Bradford assay (17). All of the oligonucleotides, the coordinates of which are given in Table 1, were purified by PAGE. For binding reactions, 5 μg of nuclear proteins were incubated with 1 μg poly(dI-dC) and 1 μg end-labeled oligonucleotide (nt 54–1) in 20 μl in the presence of 10 fmol of [32P]ATP end-labeled double-stranded probe for 20 min at room temperature. Oligonucleotide competition experiments were performed in the presence of 50–20 μl of cell extract with the dual luciferase assay system (Promega), following the manufacturer’s instructions. The luciferase activity was normalized to the renilla activity and expressed relative to the activity of the control plasmid.

RESULTS

Sp1 Plays an Essential Role in the Regulation of the Endogenous hMUC2 Gene in HT29 Cells. In previous work, we demonstrated that the expression of the human MUC2 gene could be regulated by F and TPA in HT29 cells, a human adenocarcinoma cell line (8). Upon cloning the human MUC2 promoter, we (11) and others (12) noted the presence of several GC-rich boxes which could represent binding sites for the Sp family of transcription factors. Therefore, we investigated whether Sp family members were important in the regulation of MUC2 gene expression. We analyzed the effects of mithramycin, a drug that modifies GC-rich regions of the DNA and blocks Sp1 binding (18), on the expression of MUC2 in HT29 cells induced by F and TPA (8). As shown in Fig. 1A, pretreatment with mithramycin completely abolished the accumulation of MUC2 mRNA in F- and TPA-induced HT29 cells, which strongly suggests that Sp1 binding is essential for MUC2 expression. In gel shift experiments performed in the presence or absence of mithramycin, we confirmed that the drug inhibited Sp1 and Sp3 binding to human and mouse oligos 1 to 3, described in Table 1 (Fig. 1B; and data not shown).

Cloning and Characterization of the Promoter of the Mouse Muc2 Gene. To confirm the importance of the Sp family of transcription factors in the regulation of the MUC2 gene, we extended our regulatory studies to the mouse Muc2 gene, which in the intestine has a pattern of expression similar to that of the human gene, suggesting that similar mechanisms regulate the expression of the genes in the two species (14). Therefore, we cloned and performed structural and functional analysis of the promoter of the mouse Muc2 gene, which has not been reported. A mouse 129Sv/OLA genomic library was screened with a PCR-generated mouse genomic probe corresponding to the m-1.2kb plasmid. A unique PvuII site, present in both the mouse and mouse promoters, was used to generate the 140-bp constructs by cloning the PvuII/HindIII fragment into the Smal/HindIII sites of the pGL2 vector generating the m- and h-1.4kb constructs, respectively.

The mouse mutant Sp1/AP2 plasmids were constructed with the MORPH site-directed mutagenesis kit (5 prime-3 prime, Inc.) using an oligonucleotide, spanning from nt −94 to −59, with a mutated Sp1/AP2 site (wt 5’CCGCCTCTCGG3’; mutant oligo 5’CCatCactGGA3’). The extension reaction was done in 40 μl with KCl (pH 8.3).

<table>
<thead>
<tr>
<th>Table 1 Oligonucleotides for Gel shift assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
</tr>
<tr>
<td>Oligo1 nt34−54</td>
</tr>
<tr>
<td>Oligo2 nt−103</td>
</tr>
<tr>
<td>Oligo3 nt−156</td>
</tr>
<tr>
<td>Oligo4 nt−212</td>
</tr>
<tr>
<td>Oligo5 nt−271</td>
</tr>
<tr>
<td>Oligo6 nt−340</td>
</tr>
</tbody>
</table>

"Nucleotide coordinates are given relative to the translation start site, as in Fig. 2C."
to nts 2408 to 2863 of the published cDNA sequence of the NH₂-terminal region of the rat Muc2 gene (15). We isolated two overlapping clones that comprised approximately 15 kb of the mMuc2 gene. A restriction map of the assembled clones is shown in Fig. 2A. The clones encompass the 5' end of the mMuc2 gene, including approximately 3.5 kb of 5' untranscribed region, the nt composition of which was determined; this sequence has been deposited in GenBank under the accession no. AF221746.

To determine the transcription start site of the mMuc2 mRNA and hence delineate the upstream promoter region, we used a primer extension analysis that detected a single extension product in RNA isolated from colon and small intestine but not in RNA from stomach and kidney, where Muc2 is not expressed, as shown in Fig. 3. The mMuc2 mRNA start site mapped to a G residue located at position 229 from the ATG. A similar initiation site for transcription has been reported for the rat and human MUC2 genes (15, 19).

Comparative analysis of the upstream DNA sequences of the human and mouse MUC2 genes revealed that the proximal promoter region was well conserved (homology 1 in Fig. 2B) and identified the presence of several putative recognition sequences for known transcription factors, including multiple GC-rich boxes, which may represent Sp1-binding sites. Although several of these putative binding sites were common to both the mouse and human genes, others were unique to each promoter. Most notable, differences were found in the regions surrounding the TATA box, which is well maintained (Fig. 2C). Furthermore, several additional regions well conserved between mouse and human MUC2 promoters were identified further upstream from the TATA box, and their location is shown diagrammatically in Fig. 2B.

The Proximal Promoter Region of the Mouse and Human MUC2 Genes Shows Distinct Binding Properties to Members of the Sp1 Family. To investigate whether the GC boxes identified in the mouse and human proximal region represented Sp1-binding sites, we designed oligonucleotides to be used in gel shift assays that span approximately 300 nts of the proximal promoter region. These oligonucleotides were numbered progressively 1 to 6 starting from the ATG codon, and their coordinates are given in Table 1. Fig. 4A shows the result of a representative experiment using human oligonucleotide 1, which contains a potential Sp1-binding site, and nuclear extract from HT29 cells. A lower mobility band (complex I) and two faster migrating bands (complexes II and III) were observed which could be competed specifically by 50-fold molar excess of cold oligonucleotides 1 and 3 but not of an unrelated oligonucleotide. These bands were competed as well by human oligonucleotide 2, which also harbors sequences related to the Sp1-binding site, although at lower efficiency. Oligonucleotides 4 and 5, which do not contain
Sp1-related binding sites, did not affect any complex formation. In addition, an oligonucleotide containing the Sp1 consensus binding site completely abolished the formation of all of the DNA-protein complexes (compare Lane 1 versus Lanes 2 and 7). To identify which member of the Sp1-related family was present in these complexes, we used an antibody specific for Sp1. Preincubation of nuclear extract with the anti-Sp1 antibody resulted in a specific supershift of complex I, leaving complexes II and III unaffected (Fig. 4A), which demonstrates that the upper band contained Sp1, whereas the lower bands might harbor other members of the Sp1 family (see below) as indicated by the competition experiments. An unrelated antibody (hC4d) did not affect the formation or migration of any complex.

When similar gel shift experiments were performed with mouse oligonucleotides spanning the proximal region of the mouse Muc2 promoter, we obtained similar, but distinct, results. As shown in Fig. 4B, a prominent band (complex I) was observed when mouse oligo 2 was used as a probe along with nuclear extract of HT29 cells. This band was not formed in self-competition experiments or when using oligo 1, 3, and an oligo that harbored the Sp1 consensus binding site as competitors, whereas oligo 5 showed only partial competing activity. No effect on the formation of the upper band was observed using oligos 4 and 6 as competitors. In addition, all of the oligos that showed competing activity for complex I formation were also efficient in blocking the formation of the lower band (complex II). Preincubation of the reaction mixture with antibody to Sp1 supershifted only complex I, not affecting the lower band (Fig. 4B), similar to the data obtained with the human oligos.

To ascertain whether other members of the Sp1 family were present in the additional DNA-protein complexes observed with the human and mouse oligonucleotides, which were specifically competed by the Sp1 consensus oligo, we performed gel shift experiments in the presence of an antibody that specifically recognizes the Sp3 factor. As shown in Fig. 5, the formation of complex II with human oligo 1 (Fig. 5A) and 3 (Fig. 5B) as probes was abolished by the presence of anti-Sp3 antibody in the reaction, indicating that the antibody recognizes an epitope required for DNA interaction. The formation of complex I, which contains Sp1, was unaffected. Conversely, anti-Sp3 antibody had no effect on the formation of any complexes when mouse oligos 2 and 1 were used as probes (Fig. 5A; and data not shown), but it blocked complex II formation with mouse oligo 3 as a probe (Fig. 5B).

In summary, Sp1 and Sp3 could bind to the proximal promoter region of the human MUC2 gene, a property shared only by mouse oligo 3. Conversely, the region of the mouse promoter corresponding to oligos 1 and 2 failed to bind Sp3.

**Mouse and Human Proximal Promoter Regions Display Different Functional Activity.** The different binding pattern of Sp1 and Sp3 to oligos spanning the proximal promoter region of the mouse and
Fig. 5. Differential binding of Sp3 to the mouse and human proximal promoter regions. Supershift assays were performed with the indicated mouse and human oligonucleotides using HT29 cell extract in the presence or absence of anti-Sp3 antibody in the reaction; A, shows that the addition of anti-Sp3 antibodies blocked the formation of complex II using human oligo 1 as a probe (compare Lane 3 with control Lane 1, left panel). No effect was seen when mouse oligo 2 was used as a probe (compare Lane 3 with control Lane 1, right panel); B, supershift experiment was performed with mouse and human oligo 3. Complex II formation was blocked by anti-Sp3 antibody using either h- or m-oligo 3 as probes (left panel, compare Lane 3 with control Lane 1 for the human oligo, right panel data for the mouse oligo). As control, anti-Sp1 antibody was included in all of the experiments resulting in supershift of only complex I.

The Proximal Promoter Regions of the m and hMUC2 Gene Are Equally Responsive to Overexpression of Sp1 and Sp3. Mitomycin inhibits binding of both Sp1 and Sp3, and both factors have been shown by gel shift analysis to form complexes of distinct characteristics with the proximal promoter region of the mouse and human genes (Fig. 4–5). Therefore, we investigated whether the different functional activity of the mouse and human –0.14kb reporters was a direct consequence of the different binding pattern of Sp1 and Sp3 to this promoter region in the two species by assessing the effect of Sp1 and Sp3 overexpression on human genes prompted a closer inspection of the human and mouse DNA sequences in this region. Although the homology 1 region is the most conserved between the mouse and human 5’ untranscribed DNA, there is a lack of homology in the sequence surrounding the conserved TATA box region. In the mouse promoter at nt position −80 (Fig. 2C), there is a Sp1/AP2 site, conserved in the rat Muc2 promoter but not in the human sequence, which has been reported essential for the expression of the rat Muc2 gene in SPOC cells, a tracheobronchial cell line (20). To investigate the functional significance of this difference, we cloned a short fragment from the ATG to −0.14 kb, comprising the region of interest that included Sp1-binding sites as determined by our gel shift experiments, from both the human and mouse promoters into pG52basic and compared its activity with that of reporter vectors harboring additional upstream untranscribed DNA. All of the reporter plasmids contain mMuc2 5’ untranscribed DNA regions starting at the initiation of translation (+1) and extending upstream as represented schematically in Fig. 6A. The different constructs were transiently transfected into HT29, an adenocarcinoma cell line. The m-0.14kb construct showed the same level of activity as the longer −1.2kb plasmid, which was slightly lower than that associated with the −3.5kb vector that contained all four regions of homology between the mouse and human promoter (Fig. 6B). In contrast, the human h-0.14 kb was only half as active as the longer h-0.3kb construct, which displayed maximal transcriptional activity as reported previously (11).

Thus, these results suggested that the differences present in the proximal promoter region between the mouse and human gene had functional significance. To directly investigate whether the Sp1/AP2 site, present in the mouse and absent in the human sequence, contributed to the difference observed between the mouse and human constructs, we altered it by site-directed mutagenesis to change five nts in the Sp1/AP2 site, spanning from 71 to 81 of the mouse proximal region, was mutated by site-directed mutagenesis, as described in “Materials and Methods.” Luciferase activity was measured relative to that of control pGL2basic plasmid (fold of induction) after correction for transfection efficiency, as determined by the activity of the Tk-renilla reference plasmid. The data represent the average of at least three independent experiments, each performed in triplicate. Bars represent SE.
overexpression of Sp1 and Sp3, we identified the relative contribution of individual GC boxes and of the factor they bind to the regulation of the mouse Muc2 gene, which appears to be different in the two species.

We show that oligonucleotides spanning the region from +1 to −170 of the mouse and human promoters (oligos 1–3) form complexes with Sp1. However, although the human oligos, which span this region, can also form Sp3-specific complexes, only mouse oligo 3 can bind Sp3. Both human and mouse oligo 3 contain the sequence GCCACACCCA, which was shown by Gum et al. (12) to form at least two distinct complexes, the slower one specifically containing Sp1. The pattern is very similar to both the human and mouse oligo 3, and thus the fast migrating form most likely represents Sp3/DNA complexes, as shown by our data.

Accordingly, we show that the basal level of activity of a minimal mouse promoter (m −140kb vector) is increased by both Sp1 and Sp3 overexpression, the latter having a larger effect. However, our data suggest that the mouse Sp1 is the major contributor to the regulation of the basal activity of the mMuc2 promoter and acts, at least in part, through the Sp1/AP2 site located at −80 in the mouse sequence, because mutation of the Sp1/AP2 site greatly reduces the basal activity of the m-0.14kb vector. This Sp1/AP2 site is very well conserved structurally and functionally in the rat MUC2 promoter (20) but not in the human sequence. Moreover, mutation in the Sp1/AP2 site eliminates the enhancement by Sp1 overexpression, although it only partially affects induction by Sp3 overexpression.

Finally, we show that, similar to the mouse promoter, the human proximal promoter region is activated by Sp1 and Sp3 overexpression demonstrating that the distinct binding patterns of Sp1 and Sp3 to the mouse and human proximal promoters do not directly determine their different transcriptional potency. However, our mutational analysis has identified the −80 GC box in the mouse sequence as responsible for the higher activity of the proximal promoter of mouse compared with human. Because this Sp1-binding site is intertwined with a putative AP2-binding site not conserved in the human sequence, it is possible that the anatomy of the GC box, distinct in the mouse and human, dictates the different functional properties of the proximal promoter region of the Muc2 gene in the two species. This may be due to differential physical and/or functional interactions with other transcription factors, as it has been reported for other genes.

There is growing evidence that the Sp family plays an important regulatory function during proliferation and differentiation as the level of expression of members of this family is regulated during development and in different cell types (21) and participates in the regulation of genes that are both ubiquitously expressed as well as those expressed in a tissue-specific manner (22). Furthermore, several studies have demonstrated that Sp1 mediates activation of target genes in response to exogenous stimuli (23), including oncogenes such as Ras (24) and Rb (25) and that its activity is enhanced in some tumors (26). Finally, an additional level of regulation may be exerted through the ability of Sp1 to prevent methylation of CpG islands, which are particularly enriched in GC boxes, and to alter the organization of chromatin structure (27, 28), thus establishing transcriptional competence of target genes. It is worth noting that the status of methylation of the proximal promoter of the human MUC2 gene contributes to the level of expression of the hMUC2 gene in cell lines derived from colonic tumors (29). Thus, it is tempting to speculate that in mucinous tumors the activity of Sp1 may be altered and participate in the deregulation of MUC2 expression through direct transcriptional activation, and/or establishment of transcriptional competence of the locus.

DISCUSSION

In this study, we have established the importance of the Sp family of transcription factors in the regulation of the MUC2 gene by extending our analysis of the human promoter to the mouse promoter. The mouse Muc2 promoter, which encompasses 3.5 kb of 5′ upstream untranscribed region, was cloned, and its nt composition was determined and deposited in GenBank (accession no. AF221746).

Comparative analysis of the mouse and human 5′ untranscribed region of DNA identified four nonadjacent segments that show a high degree of sequence conservation between the mouse and human promoters that may contribute to the similar pattern of expression observed for the mouse and human MUC2 gene both in vivo (14) and in vitro (this report and Ref. 11). The proximal promoter region reveals the presence of several putative binding sites for known transcription factors. Most notable is the presence of several GC-rich boxes that we show are binding sites for members of the Sp family of transcription factors. The location and composition of several of these Sp-binding sites are maintained in the mouse and human genes. In agreement, the importance of the Sp-binding sites in the expression of the MUC2 gene is clearly demonstrated by our data, which shows that mithramycin, an inhibitor of Sp1/Sp3 binding, blocks the expression of MUC2 in HT29 cells.

By a combination of gel shift analysis, site-directed mutagenesis, and overexpression of Sp1 and Sp3, we identified the relative contribution of individual GC boxes and of the factor they bind to the regulation of the mouse Muc2 gene, which appears to be different in the two species.

We show that oligonucleotides spanning the region from +1 to −170 of the mouse and human promoters (oligos 1–3) form complexes with Sp1. However, although the human oligos, which span this region, can also form Sp3-specific complexes, only mouse oligo 3 can bind Sp3. Both human and mouse oligo 3 contain the sequence GCCACACCCA, which was shown by Gum et al. (12) to form at least two distinct complexes, the slower one specifically containing Sp1. The pattern is very similar to both the human and mouse oligo 3, and thus the fast migrating form most likely represents Sp3/DNA complexes, as shown by our data.

Accordingly, we show that the basal level of activity of a minimal mouse promoter (m −140kb vector) is increased by both Sp1 and Sp3 overexpression, the latter having a larger effect. However, our data suggest that the mouse Sp1 is the major contributor to the regulation of the basal activity of the mMuc2 promoter and acts, at least in part, through the Sp1/AP2 site located at −80 in the mouse sequence, because mutation of the Sp1/AP2 site greatly reduces the basal activity of the m-0.14kb vector. This Sp1/AP2 site is very well conserved structurally and functionally in the rat MUC2 promoter (20) but not in the human sequence. Moreover, mutation in the Sp1/AP2 site eliminates the enhancement by Sp1 overexpression, although it only partially affects induction by Sp3 overexpression.

Finally, we show that, similar to the mouse promoter, the human proximal promoter region is activated by Sp1 and Sp3 overexpression demonstrating that the distinct binding patterns of Sp1 and Sp3 to the mouse and human proximal promoters do not directly determine their different transcriptional potency. However, our mutational analysis has identified the −80 GC box in the mouse sequence as responsible for the higher activity of the proximal promoter of mouse compared with human. Because this Sp1-binding site is intertwined with a putative AP2-binding site not conserved in the human sequence, it is possible that the anatomy of the GC box, distinct in the mouse and human, dictates the different functional properties of the proximal promoter region of the Muc2 gene in the two species. This may be due to differential physical and/or functional interactions with other transcription factors, as it has been reported for other genes.

There is growing evidence that the Sp family plays an important regulatory function during proliferation and differentiation as the level of expression of members of this family is regulated during development and in different cell types (21) and participates in the regulation of genes that are both ubiquitously expressed as well as those expressed in a tissue-specific manner (22). Furthermore, several studies have demonstrated that Sp1 mediates activation of target genes in response to exogenous stimuli (23), including oncogenes such as Ras (24) and Rb (25) and that its activity is enhanced in some tumors (26). Finally, an additional level of regulation may be exerted through the ability of Sp1 to prevent methylation of CpG islands, which are particularly enriched in GC boxes, and to alter the organization of chromatin structure (27, 28), thus establishing transcriptional competence of target genes. It is worth noting that the status of methylation of the proximal promoter of the human MUC2 gene contributes to the level of expression of the hMUC2 gene in cell lines derived from colonic tumors (29). Thus, it is tempting to speculate that in mucinous tumors the activity of Sp1 may be altered and participate in the deregulation of MUC2 expression through direct transcriptional activation, and/or establishment of transcriptional competence of the locus.

Fig. 7. Sp1 and Sp3 overexpression equally affects the activity of the proximal promoter region of the mouse and human MUC2 genes. HT29 cells were transfected with the mouse wt or mutant 0.14kb plasmid (m-0.14 and m-mut.0.14 kb, respectively) and the human 0.14kb vector (h-0.14 kb) in the presence or absence of 100 ng of DNA of the Sp1 and/or Sp3 expression vectors. Luciferase activity was determined as described in the legend of Fig. 6.

the m- and h-0.14kb vector activity. As shown in Fig. 7, the activity of both the wt m- and h-0.14kb reporters was stimulated by overexpression of either Sp1 or Sp3, consistent with the gel shift analysis, demonstrating that both factors can bind to the proximal promoter regions of the MUC2 gene in the two species. Stimulation by Sp3 was consistently 2–3-fold higher than that observed with Sp1 overexpression, both for the mouse and human constructs. In addition, no interference was observed when the two transcription factors were coexpressed. However, mutation of the Sp1/AP2 site in the mouse reporter abolished the ability of Sp1 to enhance the activity of the m-0.14kb vector, whereas the same mutation only partially affected the induction mediated by Sp3, thus functionally demonstrating that the GC box at −80 in the mouse DNA is an Sp1-binding site.
ACKNOWLEDGMENTS

We are grateful to Dr. Paolo Dotto, Harvard University, Boston, MA, for the gift of the Sp1 and Sp3 expression vectors and acknowledge the help of Li Shi for the preparation of plasmid DNAs.

REFERENCES


2. Pretlow, T. B., Barrow, J., Ashton, W., O’Riordan, M. A., Pretlow, T. G., Jurcisek, J., Pretlow, T. B., Barrow, J., Ashton, W., O’Riordan, M. A., Pretlow, T. G., Jurcisek, J.


'The Sp Family of Transcription Factors in the Regulation of the Human and Mouse MUC2 Gene Promoters
