Goblet cell-specific expression mediated by the MUC2 mucin gene promoter in the intestine of transgenic mice

James R. Gum, James W. Hicks, Anne-Marie Gillespie, Elaine J. Carlson, Lazlo Komuves, Satyajit Karnik, Joe C. Hong, Charles J. Epstein, and Young S. Kim. Goblet cell-specific expression mediated by the MUC2 mucin gene promoter in the intestine of transgenic mice. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G666–G676, 1999.—The regulation of MUC2, a major goblet cell mucin gene, was examined by constructing transgenic mice containing bases −2864 to +17 of the human MUC2 5′-flanking region fused into the 5′-untranslated region of a human growth hormone (hGH) reporter gene. Four of eight transgenic lines expressed reporter. hGH message expression was highest in the distal small intestine, with only one line expressing comparable levels in the colon. This contrasts with endogenous MUC2 expression, which is expressed at its highest levels in the colon. Immunohistochemical analysis indicated that goblet cell-specific expression of reporter begins deep in the crypts, as does endogenous MUC2 gene expression. These results indicate that the MUC2 5′-flanking sequence contains elements sufficient for the appropriate expression of MUC2 in small intestinal goblet cells. Conversely, elements located outside this region appear necessary for efficient colonic expression, implying that the two tissues utilize different regulatory elements. Thus many, but not all, of the elements necessary for MUC2 gene regulation reside between bases −2864 and +17 of the 5′-flanking region.

differentiation; gene expression; granular goblet cells; colon; human growth hormone reporter

The mammalian intestinal mucosa undergoes constant and rapid renewal throughout life and provides an excellent opportunity for the study of gene expression and differentiation in highly specialized cells (8). Small intestinal epithelial cells begin their existence as the daughter cells of one or a small number of stem cells located approximately four cell positions from the base of the crypts. These cells differentiate into enterocytes, Paneth cells, enteroendocrine cells, and goblet cells. Paneth cells migrate to the base of the crypts, whereas the other cell types travel upward, passing through the crypt-villus junction and ultimately transversing the entire length of the villus before undergoing a degenerative process and being sloughed into the lumen. The continuum of differentiation has been best studied in enterocytes, the most abundant cell type in the intestinal epithelium. Most enterocyte-specific gene products are first expressed as the migrating cells approach the crypt-villus junction, approximately concurrently with the cessation of cell division (for reviews, see Refs. 11 and 36). Goblet cells and enteroendocrine cells, however, are visible at lower positions in the crypts, indicating an earlier onset of differentiation. Paneth cell differentiation also occurs as the cells migrate, with the most mature cells being located near the apex of the base of the crypts. Paneth cells have considerably longer lifetimes than do the other cells of the intestinal epithelium, suggesting unique patterns of growth and differentiation for this lineage (4, 6, 32).

Much attention has been focused on identifying the factors and sequences that regulate the transcription of enterocyte-specific genes. Studies performed examining expression of promoter-reporter chimeras in transgenic mice have been particularly informational as in vivo can the exquisite patterns of differentiation found in the intestinal epithelium be completely achieved. Two fatty acid binding protein genes designated liver fatty acid binding protein (Fabpl) and intestinal fatty acid binding protein (Fabpi), as well as the sucrase-isomaltase gene (Si), have been especially well studied using transgenic systems (9, 28, 29, 34, 38). These studies have enabled the identification of regulatory sequences important for establishing and maintaining proper gradients of gene expression along the horizontal (cephalocaudal) and vertical (crypt-villus) axes of the gut for each of these genes. In addition, sequences important for suppressing inappropriate expression have been identified, as have nuclear proteins that function in regulation. These studies have not only revealed important mechanisms for the regulation of these particular genes but have also shed considerable insight on the characteristics of gut stem cells, lineage allocation, and the factors that regulate differentiation.

The present work focuses on the regulation of a goblet cell-specific gene, MUC2. MUC2 encodes a large (>5,000 amino acid residue) gel-forming mucin abundantly expressed by intestinal goblet cells and certain other mucus-secreting cells in normal, diseased, and neoplastic tissues (12, 16, 17, 35). The 5′-flanking region of the MUC2 gene has been isolated as well, and it has been shown to have promoter activity in cultured cells (13, 26, 39). It is difficult to study the factors responsible for the regulation of this gene in cultured cells, however, in part because transfectable cell lines expressing the high levels of MUC2 found in intestinal...
goblet cells are nonexistent. To circumvent this problem, we have examined the expression of a MUC2-human growth hormone (hGH) reporter-promoter construct in multiple pedigrees of transgenic mice. This has allowed for examination of MUC2 expression in fully differentiated cell types.

The results of this study indicate that elements important for the goblet cell-specific expression of MUC2 reside in the 5’-flanking region of the gene. Examination of the pattern of reporter expression has revealed, however, important differences from that of the native MUC2 gene. Moreover, the levels of reporter expression exhibited by the different pedigrees varied markedly, indicating significant integration site dependence and lending credence to the notion that there is regulatory significance to the clustering of secretory mucin genes in a single region of chromosome 11p15. These results provide experimental evidence for the importance of the proximal portion of the MUC2 gene in effecting goblet cell-specific expression, indicate major differences in the regulation of MUC2 expression in the colon and the small intestine, and provide insight into goblet cell-specific gene regulation along the crypt-villus axis of the small intestine.

**METHODS**

Construction of the MUC2-hGH transgene and transgenic mice. A construct containing bases −2864 to +17 of the human MUC2 gene 5’-flanking region spliced into the 5’-untranslated region of the hGH gene was prepared using a strategy similar to the one detailed previously for preparing untranslated region of the hGH gene (21). To generate an Xba I site at base +17 of MUC2, the PCR was used with GMUC46 as target DNA and primers 5’-TAAGGAGCTGACCCAGACTTGGTCTTGGGC and 5’-CCATGGTGCTCTAGAAGGGGGCCGTTGGG, embossed bases altered to create the restriction site. The resulting 364-base fragment, which contains the proximal portion of the MUC2 5’-flanking region, was digested with Xba I and Bsm I and ligated to a similarly digested construct containing bases −2864 to +806 of MUC2 cloned into the Sal I site of pBluescript. Digestion with Sal I and Xba I yielded bases −2864 to +17 of MUC2 for ligation into pOGH (Nichols Institute, San Juan Capistrano, CA). The portion of the sequence generated by PCR and the splice points were confirmed by sequence analysis to ensure the absence of errors. The resulting transgene containing the hGH structural gene ligated immediately downstream of bases −2864 to +17 of MUC2 (Fig. 1) was retrieved by digestion with Sal I and EcoRI for transgenic mouse production. Mice were prepared by microinjecting C57BL/6J × DBA/2 F1 hybrid zygotes with ~500 copies of the transgene according to standard protocols (20). The resulting founders were bred to C57BL/6J mice to propagate the lines. The experiments reported here were conducted using F1 or F2 mice.

DNA and RNA extraction and blot analysis. DNA was isolated from the tips of mouse tails using proteinase K digestion and phenol-CHCl3 and CHCl3 extraction followed by isopropanol precipitation (3). DNA was digested with BamHI, electrophoresed, and blotted to nylon membranes as described previously (12). The −2864 to +17 MUC2 fragment of the transgene was used as probe. The 520-bp internal BamHI fragment (Fig. 1) was used for estimation of transgene copy number by comparing the band intensities of the mouse DNA to standards prepared by digestion of known quantities of the plasmid used for transgene construction. NIH Image 1.54 software was used for densitometric intensity comparisons of autoradiograms. RNA was extracted from tissues following homogenization in Tri reagent using the protocol recommended by the manufacturer (Molecular Research Center, Cincinnati, OH). For small intestinal RNA extraction, 5-cm segments were used from the proximal, middle, and distal segments of the intestine. Colonic RNA was extracted from a 5-cm segment taken from the middle of the colon. RNA blots were prepared, hybridized, and washed as previously described. An hGH probe spanning bases 142–823 of the hGH message was prepared using the PCR and plasmid 39384 from the American Type Culture Collection as target DNA. A probe for the mouse homolog of MUC2 was created as described in text.

Fig. 1. MUC2-human growth hormone (hGH) transgene. The 5’ portion of the MUC2 gene is represented at top. Exons of MUC2 structural gene are depicted as solid blocks and numbered; sequenced portion of 5’-flanking region contained in clone GMUC46 is also shown. Thin horizontal line below depicts the transgene. Nar I site was converted to Sal I site, and Xba I site was introduced as described in text. Solid blocks represent the 5 exons of the hGH structural gene. At bottom, sequence of MUC2 surrounding transcription start site is given. Bent arrow represents start of transcription, and underlined ATG is initiation codon. Final sequence is that of the transgene. Bolded bases represent non-MUC2 sequence. Specifically, 3 bases were altered to create Xba I site for doing into pOGH, and sequence from BamHI I site to initiating ATG is part of the 5’-untranslated region of the hGH gene.
spanning bases 1573–1912 of the rat sequence (30) was also made using the PCR with reverse-transcribed mouse colonic RNA as target DNA. This 340-base probe, designated D2, had ~96 and 83% sequence similarity to the rat and human sequences, respectively. The probe for glyceraldehyde-3-phosphate dehydrogenase was described previously (15).

RNase protection analysis. Two constructs were prepared for templates for RNA probe preparation using the PCR with the transgene-containing plasmid as target DNA. These constructs both spanned the MUC2-hGH splice junction and both terminated at base 27 of the first intron of the hGH gene. Construct F1 initiated at base -170 of the MUC2 5'-flanking sequence and construct F2 initiated at base -43. The appropriate fragments were cloned into pBluescript (SK-–) and sequenced to ascertain that point mutations were absent. Pst I digestion was used to yield templates for antisense probe preparation using T7 RNA polymerase. Hybridization, digestion, electrophoresis, and analysis were performed as previously described using 20 µg of total RNA from distal small intestine or colon (17).

Immunohistochemistry. Mice were anesthetized using methoxyflurane and killed by cervical dislocation. Tissues were removed rapidly, rinsed in phosphate-buffered saline, fixed in Bouin’s solution, and embedded in paraffin before the cutting of 5-µm sections. Rabbit polyclonal anti-hGH (1:500, Dako, Carpinteria, CA) was used as the primary antiserum in an overnight incubation as described by Markowitz et al. (29), followed by detection with immunogold silver staining using reagents purchased from Zymed Laboratories. The silver enhancement procedure was performed either one or two times, depending on antigen level in a given tissue. Slides were counterstained using Alcian blue. Double labeling using anti-hGH and immunogold silver staining followed by rabbit anti-serotonin (1:3,000; Incstar, Stillwater, MN) and FITC-conjugated pig anti-rabbit IgG (Dako) was also performed as described by Markowitz et al. (29).

Immunoelectron microscopy. Reagents for electron microscopy (glutaraldehyde, paraformaldehyde, sodium cacodylate, uranyl acetate) were purchased from Electron Microscopy Sciences (Fort Washington, PA). LR Gold resin was obtained from Polysciences (Warrington, PA). Tissue samples were fixed in room temperature by immersion in 2% paraformaldehyde and 0.2% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.20). After 2 h of fixation at room temperature, the samples were washed overnight in buffer. Tissues were embedded into LR Gold resin, polymerized at –20°C by ultraviolet light (2, 24). Thin (80 nm) sections were cut on an RMC MT-7 ultramicrotome with a diamond knife and collected on nickel grids (Polysciences).

For immunogold detection of the hGH reporter, nonspecific binding of the immunoreagents was prevented by incubating the grids for 30 min on a drop of blocking buffer: 10 mM Tris buffer (pH 7.40) containing 2% bovine serum albumin, 0.5% cold-water fish skin gelatin, 0.1% Tween 20, and 500 mM sodium chloride. The grids were then incubated with the Dako primary antibody (135 µg/ml) in a humid chamber at room temperature for 12 h, followed by extensive washing with blocking buffer (5 times, 5 min each). The antibodies bound to the sections were detected by goat anti-rabbit IgG-Au10 (Aurion, Wageninin, The Netherlands) for 30 min, followed by extensive washing: three times with blocking buffer (5 min each), three times with 10 mM Tris (pH 7.40) containing 500 mM sodium chloride, and three times with distilled water. Controls included 1) omission of the primary antibody; 2) incubation with a nonrelevant antibody, i.e., rabbit anti-porcine IgG in lieu of the specific antiserum; 3) incubation with a nonrelevant gold reagent, i.e., goat anti-mouse IgG-Au10 in lieu of the specific gold reagent; and finally 4) labeling of nontransgenic, control tissues. All of the controls resulted in negligible deposits of colloidal gold over the sections, indicating specific binding of the anti-hGH antibody. The sections were contrasted with 2% osmium tetroxide for 15 min, washed with distilled water, and stained with Sato’s lead stain. The dried grids were then coated with 0.25% Formvar (24) and examined and photographed using a Zeiss 10CR electron microscope at 60 kV.

RESULTS

Analysis of transgenic mice. DNA blot analysis indicated the presence of transgene(s) in seven founder mice. All of these mice were fertile, and all propagated the transgene(s) to their offspring in an autosomal dominant pattern. One of the mice, founder 6, apparently incorporated transgenes in two different genomic locations because its offspring exhibited different patterns on DNA blot analysis. The two genotypes segregated in the F1 generation and were designated lines 6 and 6’ (Fig. 2). Thus a total of eight transgenic lines were produced and available for analysis. All transgenic mice resembled their normal littermates in appearance.

Figure 2 shows a blot containing BamHI-digested DNA from different lines probed with the MUC2 portion of the transgene. As indicated on the map shown in Fig. 1, the transgene contains two internal BamHI fragments of 520 and 331 bp. These two fragments were present at various levels in each transgenic line. Densitometric analysis of the 520-bp fragment indicated the transgene to be present at 1 to ~32 copies per diploid genome in heterozygotes (Table 1). The pattern exhibited by the external BamHI fragments varied with line. These fragments hybridize to the region of the probe located between the SalI site and the first BamHI site. The 231-bp fragment is a unique fragment in line 6’.
of the transgene (Fig. 1). Lines 3, 5, 6’, and 8 have only a single copy of transgene, and they exhibit external fragments of various sizes. This variation is as expected because the sizes of the external fragments are dependent on the locations of BamH I sites in the transgene integration sites of the individual lines. Concatemeric insertion of multiple copies of transgene would give a 4,200-bp fragment because this is the size of the hGH reporter, which initiates from a BamH I site, plus the portion of the MUC2 gene 5’-flanking sequence proximal to the first BamH I site included within this sequence (Fig. 1). This appears to have occurred with lines 1 and 2. Lines 4 and 6 have transgene copy numbers of ~3 and 4, respectively, but do not appear to have undergone concatemeric insertion because they lack the 4,200-bp fragment seen in lines 1 and 2. The mode of insertion in these two lines is therefore unknown at present.

Preliminary experiments indicated expression of the hGH reporter gene in the distal small intestine of several lines. This was examined systematically using RNA blot analysis, and expression of the 800-base hGH message was detected in four of the eight lines (Fig. 3). Expression was observed in the stomach and heart of line 4. No expression was observed in the liver, kidney, or brain of any line.

An important test of the fidelity of a promoter/reporter model to the endogenous promoter is whether transcription is initiated from the same position on the promoter in both instances or not. To examine this, RNase protection assays were performed to map the transcription start sites of the transgene. Figure 5 depicts an experiment in which RNA from the distal small intestine and colon of line 1, 2, and 4 mice together with a nontransgenic control was examined. Two separate riboprobes, both terminating at the same nucleotide in the first intron of the hGH reporter but initiating at different points in the MUC2 promoter, were used. Two major fragments of 95 and 98 bases in length and several minor fragments were obtained with both probes when line 1 or line 4 distal small intestine RNA was used (Fig. 5). Line 2 distal small intestine RNA yielded faint but detectable fragments in

| Table 1. Transgene copy number in the various lines of MUC2-hGH mice |
|-----------------|-------------|
| Line | Copy Number |
| 1   | 32          |
| 2   | 5           |
| 3   | 1           |
| 4   | 3           |
| 5   | 1           |
| 6   | 4           |
| 6’  | 1           |
| 8   | 1           |

hGH, human growth hormone.

Table 2. Expression of hGH reporter in the various transgenic lines

<table>
<thead>
<tr>
<th>Line</th>
<th>Normalized Expression of hGH, %</th>
<th>hGH Expression per Transgene Copy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>13.7</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>6’</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Expression of hGH in distal small intestine total RNA was determined by densitometric analysis using NIH Image software. Normalized expression levels were divided by transgene copy number (Table 1) to give hGH expression per transgene copy.

Fig. 3. RNA blot analysis of transgene expression in all lines. Blot containing distal small intestine total RNA samples probed for hGH message expression is shown at top. Blot was later analyzed using a glyceroldehyde-3-phosphate dehydrogenase (GAPDH) probe to ensure that sample was loaded in each lane.
the same positions, the expected result given the low level of transgene expression in this line (Fig. 3). The sizes of these fragments position the major start sites at bases 227 and 230 of the MUC2 promoter sequence. This is in good agreement with the start sites observed for endogenous MUC2 transcription in the human colon, which exhibited four major start sites between nucleotides 223 and 227 (17). The nontransgenic control RNA produced no protected fragments. The fact that both probes yielded the same size fragments provides evidence that the 5′-termini were correctly approximated, because both probes are protected to the same point on their 3′-terminus, i.e., to the end of the first exon. When colonic RNA from line 2 was used, the same two 95- and 98-base fragments were faintly visible with probe F1, but not with probe F2 (Fig. 5). This is probably due to differential sensitivity of the assay with the two probes. The other lines did not exhibit bands with colonic RNA, again the expected result because they express reporter minimally, if at all, in this tissue (Fig. 4).

Table 3. Normalized MUC2 gene expression in the proximal, middle, and distal small intestine and colon

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normalized Expression, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal SI</td>
<td>10.1 ± 9.2</td>
</tr>
<tr>
<td>Middle SI</td>
<td>22.0 ± 14.6</td>
</tr>
<tr>
<td>Distal SI</td>
<td>29.2 ± 16.4</td>
</tr>
<tr>
<td>Colon</td>
<td>100</td>
</tr>
</tbody>
</table>

Normalized expression is given as mean ± SD. Expression of MUC2 in 6 mice of various lines was estimated densitometrically from RNA blots using NIH Image software. SI, small intestine.

Cell type specificity of transgene expression. Immunohistochemical analysis was used to assess the cell type specificity of reporter expression in the various trans-
genic lines. Figure 6A shows a longitudinal section of the distal small intestine of a line 1 mouse. It is clear that transgene reporter expression colocalized with the Alcian blue-stained goblet cells. The silver particles were deposited over the rough endoplasmic reticulum and Golgi-containing region of the cells as can best be seen examining villi cut in cross section (Fig. 6B). Close examination of the sections reveals that transgene expression begins deep in the crypt, far below the crypt-villus junction. Thus transgene expression occurs as an early event, beginning approximately with differentiation into the goblet cell lineage. It is also clear that enterocytes, which constitute the major cell type present in the small intestinal epithelium, do not express transgene. Furthermore, some goblet cells from line 1 mouse intestine also fail to express transgene, as can be seen in both Fig. 6A and Fig. 6B. A similar pattern of expression can be seen with line 4 mice, although the level of expression in this line is considerably lower (Fig. 6, C and D). Again, the staining of goblet cells beginning at positions deep in the crypts is observed. No hGH reporter expression was observed with non-transgenic mouse intestine (Fig. 6E). Attempts to localize transgene expression in line 2 and line 6 mouse intestine using this technique were unsuccessful, presumably because hGH protein levels were below the limits of detection (not shown).

The intestinal epithelium contains small numbers of enteroendocrine cells interspersed among larger numbers of enterocytes and goblet cells. The immunohistochemical analysis described above suggested goblet cell-specific expression of reporter but could not rule out the possibility of transgene expression in enteroendocrine cells, which occur only infrequently. To examine this, sequential double labeling of cells in tissue sections was performed using anti-hGH detected with immunogold silver staining and anti-serotonin detected with FITC-labeled second antibodies. Serotonin-producing enteroendocrine cells represent the majority of enteroendocrine cells produced in the mouse distal
small intestine (32). Examination of the double-labeled sections revealed segregation of the labels into different cell types; i.e., coexpression of the two labels in the same cells was not observed. Illustrative of this experiment, Fig. 7 shows three serotonin-producing enteroendocrine cells that lack reporter expression. Thus these results indicate that detectable levels of transgene reporter are not expressed in serotonin-producing enteroendocrine cells.

Ultrastructural localization of transgene expression. We expanded our examination of transgene expression in the intestine to the ultrastructural level using immunoelectron microscopy. These studies confirmed and extended our light microscopic observations. Electron microscopy revealed no apparent ultrastructural differences in the distal small intestinal mucosa of control vs. transgenic animals (Fig. 8, A–C). In control animals, no hGH-reactive cells were found. Line 1 distal small intestinal mucosa, however, contained a subset of goblet cells with hGH-reactive, electron-dense secretory granules (Figs. 8, C and D. and 9). No other cells, including Paneth cells (Fig. 8B), enteroendocrine cells, or enterocytes, were noted that contained detectable hGH reporter. Closer examination of the ultrastructural characteristics of the goblet cells revealed that the hGH-reactive, small, electron-dense granules were located within the mucus globules (Figs. 8, C and D. and 9. A–D). The mucus globules themselves did not contain any hGH-reactive material. These ultrastructural features indicate that the hGH-expressing goblet cells are identical to the so-called granular goblet cells described earlier by Cheng (7). No intracellular organelles, including the endoplasmic reticulum, Golgi apparatus, or mucus globules, contained any hGH reactivity in common goblet cells, which lack the dense granules (Fig. 8A), the identifying feature of granular goblet cells. Whereas hGH-reactive material was not detected in the endoplasmic reticulum of granular goblet cells, it was clearly seen in the trans-Golgi network, where the label was associated with vesicles of different sizes (Fig. 9. A and B). The detection of reporter in the Golgi but not the endoplasmic reticulum probably indicates a higher reporter concentration in the former organelle, possibly as a result of a packaging process. Label was also found in the presumably nascent dense granules associated with the trans-Golgi (Fig. 9C). In the mature granules the label was seen both at the granular surface and in the matrix. Secretion, i.e., exocytotic release of the granules, was observed. However, no hGH-reactive material was seen in the mucus, possibly because of degradation or dilution of the epitope in the intestinal lumen.

DISCUSSION

Highly glycosylated, polymeric, secreted mucins such as MUC2 serve to coat and protect the gastrointestinal, reproductive, and respiratory tracts. Given their role in protection, large size, and the complex processes required for their biosynthesis, it is not surprising that alterations in mucins, and their levels of expression, are associated with several diseases. In certain cancers, mucin oligosaccharides are altered in several ways, including a reduction in number, shortening, the exposure of normally cryptic inner chain residues, and the expression of novel carbohydrate structures (21, 23). The levels and types of mucins expressed are altered in cancers as well. For example, human cancers, especially colon cancers, differ widely in the levels of MUC2 they express (18). Most colon cancers express only low levels of MUC2 compared with normal colon, but a class known as mucinous colon cancers expresses high levels of MUC2, suggesting that they follow a different mutational pathway to malignancy (19). Several diseases of the airways are associated with altered mucin gene expression as well (22, 33). These include cystic fibrosis, in which Pseudomonas infection appears to induce MUC2 expression, perhaps contributing to airway obstruction (26). This association of altered mucins with disease has led us to examine in detail the structure and function of mucins and the factors that control their expression.

We and others have characterized MUC2 gene expression in vitro using transient transfections of cultured cells with promoter/reporter constructs and nuclear protein binding studies. This work has identified a CACCC motif located between bases −88 and −80 of the MUC2 reporter that binds Sp1 and related factors and that appears to be important functionally because its presence establishes a low level of transcription in all tested cells whether they express MUC2 or not (13). We have also identified bases −228 to −171 as possibly being important in conferring cell-type specificity on
the MUC2 promoter and a nuclear factor-κB site located between bases −1452 and −1441 that participates in the induction of MUC2 by Pseudomonas (13, 26, 27). It is important to examine the expression of MUC2 in vivo, however, because tissue culture systems lack high-level expression and authentically differentiated cell types, essential components of cell type-specific gene expression. Previous studies using transgenic systems have shed considerable insight into the factors that control gene regulation in enterocytes, enteroendocrine cells, and Paneth cells (4, 11, 25, 36). In this work we extend the examination of intestinal gene expression in vivo to the fourth cell lineage, i.e., goblet cells.

Tissue-specific expression of the MUC2-hGH transgene. The MUC2-hGH transgene is expressed at high relative levels in the distal small intestine of four of the eight lines obtained (Figs. 3 and 4). High levels of reporter expression in the middle small intestine are also observed in three lines. On the contrary, very low expression levels were observed in the proximal small intestine and significant expression in the colon was observed only in line 4. This contrasts with endogenous MUC2 gene expression, which follows an increasing gradient along the horizontal axis of the intestine (Table 3). Also, significant transgene expression was not observed in the non-MUC2-expressing tissues examined. This complex pattern of expression indicates that many elements function in the control of MUC2 levels in tissues, as has been observed in experiments examining the promoters of other intestinal genes in transgenic mice (9, 34, 38).

Transgene expression in the distal small intestine indicates that elements required for expression in this tissue are located between bases −2864 and +17 of the MUC2 5′-flanking sequence. Clearly, however, this expression is influenced by the local genetic environment dictated by insertion site because four of the eight lines expressed no detectable levels of reporter and in the other lines there was wide variation in the level of reporter expressed when normalized to copy number of transgene incorporated (Table 2). Marked integration site dependence for transgene expression was observed using bases −201 to +54 of the Si promoter but not...
when the −8500 to +54 sequence was used (38). This result was attributed to the ability of the region between bases −8500 and −201 to insulate the transgene from surrounding chromatin effects. In this regard, it is interesting to note that MUC2 resides within a 400-kb region of chromosome 11p15.5 that contains at least three other secretory mucin genes (31). There may be regions within this "MUC cluster" that have insulator activity or even that function in the regulation of several mucin genes. The lack of even moderate levels of reporter expression in all tested non-MUC2-expressing tissues suggests the possibility that at least one silencer located between bases −2864 and +17 of the MUC2 5′-flanking sequence is an important component in achieving tissue-specific regulation.

The fact that reporter is expressed in the colon in only one transgenic line is especially surprising. This result lies in stark contrast to what is observed in the small intestine of the same animals and strongly suggests that different combinations of nuclear factors regulate MUC2 expression in the small intestine and colon. One possibility is that elements found elsewhere than on the −2864 to +17 region of MUC2 are utilized by colon-specific factors to produce high-level expression. Possibilities include sequences found upstream of base −2864, in introns, or even in the 3′-flanking sequence. Experiments involving other intestinal genes suggest that multiple enhancers and silencers function in concert to effect tissue-specific expression (11, 36). The similar locations of the transcription start sites of the transgene in mouse intestine (Fig. 5) and endogenous MUC2 in human colon indicate a common utilization of basal transcription factors by the MUC2 promoter in human and mouse intestinal cells, an expected result because several hundred bases of the proximal portion of the MUC2 promoter are conserved between human and mouse (14). The observation that one of the lines did express reporter at approximately equal levels in the colon and small intestine is again suggestive of the notion that the local genomic environment plays an important role in MUC2 gene regulation, and perhaps this effect is more important for colonic expression than for small intestinal expression.

Cell type-specific expression of the MUC2-hGH transgene. Immunohistochemical analysis indicates that the hGH reporter is expressed in the goblet cells of the...
small intestine (Fig. 6). Colocalization of hGH antigen with Alcian blue-stained mucus goblets occurs deep in the crypts, indicating a close temporal relationship between the initiation of transgene expression and the development of the goblet cell phenotype. The message for human MUC2 is also expressed deep in the crypts (1, 5). Thus transgene expression initiates in a similar early position along the crypt-villus axis as does endogenous MUC2 expression. This implies that elements required for this important aspect of MUC2 gene regulation appear to be located between bases −2864 and +17. As the goblet cells migrate past the crypt-villus junction, immunohistochemical staining of reporter becomes less pronounced and some Alcian blue-staining cells are clearly negative for reporter expression. This is in apparent contradiction to previous in situ hybridization studies that indicate that MUC2 expression persists the entire lifetime of goblet cells in the human small intestine (1, 5). It is possible, however, that this apparent discrepancy results from differences between the translational efficiencies of the messages and/or the processing and secretion of hGH and MUC2 rather than reflecting true differences in transcriptional regulation. It is also possible, however, that regulatory sequences required for continued high-level reporter expression in the villi are found elsewhere than on the portion of the MUC2 5′-flanking sequence used in transgene construction.

The absence of reporter expression in small intestinal epithelial cells other than goblet cells suggests either that silencers that suppress MUC2 expression in inappropriate epithelial cell types reside in the −2864 to +17 region or that goblet cell-specific enhancers reside in this region. The cell type specificity observed is analogous to similar transgenic models using the cryptdin 2 gene promoter and the glucagon promoter, which exhibit expression limited to Paneth cells and enteroendocrine cells of the adult mouse small intestine, respectively (4, 25). It contrasts, however, with transgenic models using the promoters of enterocyte specific genes Si, Fabpi, and Fabpl, in which reporter was detected in most epithelial cell types (11, 36). These results suggest fundamental differences between gene regulation in enterocytes and the other less-abundant cell types of the intestinal epithelium.

Previous electron microscopic analysis of the mouse small intestine revealed the presence of two types of mature goblet cells, designated common and granular mucus cells (7). These cells are similar in size and shape and are distinguished by the presence of electron-dense granules of 200–500 nm that are located within the mucus globules of granular mucus (goblet) cells. Approximately 23% of the goblet cells of the distal small intestinal crypts are granular goblet cells, the remaining being of the common variety. As the cells migrate to the villi, granular goblet cells become much less common without any evidence of a degenerative process, leading to the hypothesis that they become common goblet cells (7). In this study, we examined hGH reporter expression by immunoelectron microscopy (Fig. 8). Here, we found reporter expression only in granular goblet cells. Other cell types of the small intestinal epithelium, including common goblet cells, were not labeled. The limitation of hGH reporter protein expression to a subclass of goblet cells raises several interesting possibilities. First, transgene expression could actually be limited to granular goblet cells; i.e., elements found elsewhere than on the −2864 to +17 region may be required for expression in common goblet cells. This hypothesis is discredited considerably by the large numbers of villi goblet cells observed labeled by anti-hGH in typical histological sections. It is also possible that granular goblet cells differ metabolically from common goblet cells. This could lead to the detection of hGH by immunoelectron microscopy only in granular goblet cells, with both classes expressing antigen when examined using conventional immunohistochemistry, the more sensitive of the two techniques.

Rapid secretion of the foreign protein hGH vis-à-vis MUC2, which is typically stored by intestinal goblet cells before release, may be a factor in this regard as well. A study utilizing transgenic mice harboring an Fabpl-hGH transgene is especially relevant to our observations here (37). In this earlier study, similar immunoelectron microscopic techniques were used and expression was observed in enterocytes, enteroendocrine cells, Paneth cells, and granular goblet cells. Common goblet cells were the only small intestinal epithelial cell type without hGH antigen detectable by immunoelectron microscopy in the Fabpl-hGH mice (37). This work using a promoter with broad specificity for expression in small intestinal epithelial cells suggests that some unique feature of common goblet cells prevents the immunoelectron microscopic detection of hGH antigen in this cell type. Also, this earlier study indicates that hGH antigen is detectable in enteroendocrine cells. This suggests further that the inability to detect hGH by immunoelectron microscopy in these cell types in the MUC2-hGH mice is a result of cell type-specific expression. Additional work will be necessary to investigate the interesting observation that common goblet cells lack hGH expression detectable by immunoelectron microscopy.

This work was supported by the Department of Veterans Affairs Medical Research Service, a VA-DOD Merit Research Award, and by the University of California at San Francisco Cystic Fibrosis Core Facility, funded by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-47766 to C. J. Epstein.

Address for reprint requests and other correspondence: J. R. Gum, Jr., Gastrointestinal Research Laboratory (151M2), 4150 Clement St., San Francisco, CA 94112 (E-mail: jgum@naestrom.uscf.edu). Received 27 August 1998; accepted in final form 17 November 1998.

REFERENCES
GLOBET CELL-SPECIFIC EXPRESSION IN TRANSGENIC MICE

17. 18.
Gum, J. R., J. W. Hicks, N. W. Toribara, E. M. Rothe, R. E.
Hogan, B., R. Beddington, F. Constantini, and E. Lacy.