GUANYLIN IS AN ENDOGENOUS ligand for the intestinal cell receptor guanylyl cyclase C (GC-C). On binding to GC-C, guanylin is thought to activate a cGMP-mediated second messenger system culminating in chloride and bicarbonate efflux through the cystic fibrosis transmembrane regulator (CFTR) (7). Current understanding of the physiological function of guanylin as a mediator of chloride secretion is largely based on its similarity to heat-stable enterotoxin (STa), a peptide secreted by enterotoxigenic *Escherichia coli* and a potent agonist of the GC-C receptor pathway (11, 12, 18).

Guanylin mRNA has been detected throughout the gastrointestinal tract in a proximal-to-distal gradient, with the majority of signal residing in the terminal ileum and proximal colon (4, 37). Cell-specific localization has demonstrated that guanylin mRNA and/or immunoreactive peptide is present in numerous cell types, including the surface villous enterocytes and goblet cells (4, 6, 23, 24), Paneth cells (4), and enteroendocrine cells (2, 16). Although the regulatory properties of guanylin secretion have been examined in detail in the rat colon (28, 29), the pattern of guanylin secretion and regulatory mechanisms in individual cell types have not been specifically elucidated.

Guanylin is synthesized by intestinal epithelial cells as the 116-amino-acid precursor proguanylin. The active peptide is then liberated by cleavage of the 15–16 amino acids at the COOH-terminal portion of the propeptide (14). Guanylin is thought to exert its effect in the intestinal lumen on the apically oriented GC-C receptor. There is also a circulating fraction of proguanylin, suggesting a function of guanylin or another portion of the prohormone at a site distinct from the intestine. Although basolateral secretion of guanylin in the rat colon has been shown (28, 29), basolateral secretion in human colonic epithelium has not been verified. Proguanylin has, however, been isolated from the plasma (31) and in concentrated dialysate of patients with end-stage renal disease (20).

Our purpose was to examine proguanylin secretion through the establishment of an in vitro model in a population of cells resembling the villous epithelial enterocyte. We hypothesized that proguanylin would be produced and secreted from the apical and basolateral cell surfaces in C2/bbe1 cells grown as confluent monolayers on Transwell inserts. In addition, we hypothesized that this model could be used to study feedback inhibition as a potential regulatory mechanism of proguanylin synthesis and secretion.

GUANYLIN secretion and the role of negative-feedback inhibition in a villous epithelial cell line

JEFFREY A. RUDOLPH, JENNIFER A. HAWKINS, AND MITCHELL B. COHEN

*Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Children's Hospital Medical Center, Cincinnati, Ohio 45229*

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cells. Total RNA was isolated from zCaco-2 cells or C2/bbe1 cells that were grown in 75-cm² flasks under the conditions described above. Cells were washed once with calcium-free PBS and pulverized in guanidine isothiocyanate solution, and RNA was extracted using the method described by Chomczynski and Sacchi (3). Total RNA (30 μg) was electrophoresed on 1.5% agarose-1.5% formaldehyde gels and transferred to nylon membranes (Magnagraph, MSI, Westboro, MA). Blots were hybridized with a 32P random-labeled probe for the full-length human proguanylin cDNA (pMON22305) (17). Proguanylin quantity was quantified by the Phosphorlmaging system with Imagequant software (Molecular Dynamics, Sunnyvale, CA) and internally standardized by comparison with 18S ribosomal RNA hybridized to a 32P end-labeled probe for 18S rRNA (27).

Proguanylin detection in Caco-2 cells. Proguanylin secretion was analyzed in six-well culture plates by Western blot analysis. On day 14, fully supplemented DMEM was replaced by 2 ml of serum-free medium. A 1-ml aliquot, or 50% of the total preincubation volume of spent medium, was concentrated and purified with the modification of a previously described method (7) using octadecyl carbon columns (Waters, Milford, MA) and elution of the proguanylin with 60% acetonitrile-40% 0.01 M NaOAc, pH 5.8. For experiments using cell homogenates, washed monolayers were used after spent medium was collected. Cells were homogenized in Tris-mannitol buffer (2 mM Tris base, 50 mM mannitol, pH 8.0) and interned standardized by comparison with 18S ribosomal RNA hybridized to a 32P end-labeled probe for 18S rRNA (27).

Specificity of the antisera to proguanylin secreted in the media of C2/bbe1 cells was validated utilizing competitive binding to the fusion protein. Western blots were evaluated that contained duplicates of fusion protein (5 ng) and pooled C2/bbe1 spent medium that was purified and concentrated as described above. Before immunoblotting, the primary antibody solution containing Rb 4696-4 was preincubated with various concentrations of fusion protein. This preincubation mix was then used in the immunoblotting protocol as described above.

Transwell insert model of proguanylin secretion. The Transwell insert system was modeled as previously described by Hidalgo et al. (15). Briefly, C2/bbe1 cells were seeded at a density of 2 × 10⁶ cells/cm² onto Isopore track-etched polycarbonate membranes with a pore size of 3.0 μm (Millipore, Bedford, MA) resting in standard six-well culture plates. To confirm the existence of a tight monolayer, a subset of day 14 cells growing on the inserts was fixed at room temperature in 3% glutaraldehyde and embedded into LX112 polymer. Thin sections were cut and stained with uranyl acetate and lead citrate and examined under a Zeiss 110 transmission electron microscope. For the remainder of the inserts, day 14 cells were washed in calcium-free PBS and serum-free DMEM (2 ml) with 0.5 mg/ml of Lucifer yellow (346 g/mol; Sigma) was added to the apical side of the insert. Serum-free DMEM (2 ml) was added to the basolateral side, and the cells were incubated in standard conditions overnight. Spent media from the apical and basolateral compartments were collected and analyzed for proguanylin by Western blot analysis as described above.

To confirm functional compartmentalization, 12 μl of spent medium were diluted in 3 ml of distilled H₂O, and the relative fluorescence was determined using a spectrofluorometer (model LS50B, Perkin-Elmer, Norwalk, CT) at an excitation wavelength of 430 nm (bandwidth 5 nm) and emission wavelength of 540 nm (bandwidth 10 nm). Concentrations of Lucifer yellow were determined from standard curves that were linear in the range of concentrations tested. Medium was used for subsequent analysis only when <5% transmission occurred, suggesting a tight monolayer. In other epithelial systems, minimal transmission of Lucifer yellow has been shown to occur via fluid phase endocytosis (10), and this is postulated to occur in Caco-2 cells (15).

Modifier effect on proguanylin message and secretion. C2/bbe1 cells were subcultured in 75-cm² flasks or six-well plates and incubated for 14 days. Fully supplemented DMEM was exchanged for serum-free DMEM alone or with a potential modifier of the GC-C signaling pathway [500 nM STa, 250 μM 3-isobutyl-1-methylxanthine, 100 μM 8-bromo-cGMP (8-BrcGMP), 100 ng/ml cholera toxin (lot 10150BL, List Biological Laboratories, Campbell, CA), 100 μM glibenclamide (Research Biochemical International, Natick, MA), 2 mM thapsigargin, or 0.1 μM phorbol 12-myristate 13-acetate (PMA)]. Spent media and cells were harvested, and protein and RNA expression was analyzed as described above.

Statistical analysis. For guanylin mRNA quantitation studies, results were compared using the Friedman test, a nonparametric analog of analysis of variance, to determine that each data set correlated and to verify reproducibility of results (http://www.fon.hum.uva.nl/Service/Statistics.html). For comparison of each individual modifier with its control, the Wilcoxon matched-pairs signed-ranks test was used as a nonparametric means to determine significance. P ≤ 0.05 was considered significant.

Reagents. Reagents not specifically indicated were obtained from Sigma. All tissue culture media and supplies were obtained from Becton-Dickinson (Franklin Lakes, NJ).
RESULTS

Determination of proguanylin message and secretory product in Caco-2 cell lines. Two Caco-2 cell lines were screened for the ability to inherently express proguanylin mRNA and secreted protein. Previously, nonpolarized zCaco-2 cells were shown to produce guanylin message after confluence (17). The C2/bbe1 cell line was screened because of the superior ability of these cells to attach to a porous support and form an intact, functional barrier (30). C2/bbe1 cells, similar to the zCaco-2 cell line (17), produced a stronger hybridization signal of proguanylin mRNA per 30 μg of total RNA loaded as they became confluent (Fig. 1A). At 14 days, C2/bbe1 cells repeatedly produced a stronger hybridization signal for proguanylin mRNA and secreted more propeptide than zCaco-2 cells (Fig. 1, B and C).

Validation of antibody specificity for the proguanylin secretory fraction in spent media. The polyclonal antiserum derived from New Zealand White rabbits injected with the polyhistidine-tagged fusion protein was validated by preincubating the antiserum with different concentrations of the fusion protein before immunoblotting (Fig. 2). Control blots in which no fusion protein was added and the spent media were not quenched using the preincubation mixtures, were also detected in the spent media. These bands were not quenched using the preincubation mixtures, signifying nonspecific detection by the secondary antibody or chemiluminescence reagent (data not shown).

Morphological and functional assessment of the Transwell insert system. To study the secretory pattern of proguanlyin, we used the Transwell insert system. Transwell inserts were seeded with C2/bbe1 cells and incubated for 14 days to allow the development of tight epithelial junctions (15). The presence of a monolayer was confirmed by transmission electron microscopy; a representative section is shown in Fig. 3. The C2/bbe1 cells appeared to form a polarized monolayer with numerous microvilli facing the apical chamber. Tight junctions were also present between cells at the apical-basolateral interface (Fig. 3, inset). To confirm that there was minimal transmigration of cells through the pores of the membrane, inserts were examined after the apical side of the membrane was scraped free of cellular matter. The basolateral side of the membrane and the base of the six-well plate were examined after staining and were devoid of transmigrating cells (data not shown). Taken together, these data confirm the morphological presence of a polarized monolayer across the insert membrane with no detectable transmigration of cells through the pores of the membrane.

Fig. 1. Proguanylin mRNA and protein expression in 2 substrains of the Caco-2 cell line. A: Northern blot of C2/bbe1 cell line as a function of time after seeding. RNA was collected at days 3 (d3), 5 (d5), 7 (d7), 10 (d10), 14 (d14), and 21 (d21) and hybridized to the 32P-labeled guanylin and 18S probes. Cells were confluent by day 7 and used in the insert model at day 14. B: Northern blot of 3 independent samples of zCaco-2 and C2/bbe1 cell RNA 14 days after seeding. Western blots are shown for 3 independent samples of spent media from cells grown for 14 days. Spent media were collected after overnight incubation and immunoblotted with Rb 4696-4 antiserum.

Fig. 2. Competitive binding assay of polyclonal antiserum to the proguanylin fusion protein. Before immunoblotting with Rb 4696-4, the antiserum was preincubated for 30 min with separate concentrations of fusion protein that approximated 0 M (A), 0.5 M (B), and 10 M (C) equivalent doses of primary antibody. Immunoblotting proceeded as described above. Signal for fusion protein (lanes 1 and 2) and purified spent media (lanes 3 and 4) decreased as the preincubation concentration of fusion protein increased, suggesting specificity of antiserum to the fusion protein and to the pooled purification product of spent media.
A functional assessment of the monolayer was performed using a Lucifer yellow exclusion assay (15). Lucifer yellow equilibrated across the membrane alone with equal distribution as assessed by fluorometry at 4 h. In contrast, with a mature monolayer of cells, minimal Lucifer yellow (<5%) appeared in the basolateral compartment after overnight incubation. The Lucifer yellow exclusion assay was performed on all inserts used in experiments to confirm a tight monolayer before evaluation for the presence of proguanylin.

**Proguanylin secretion in the Transwell insert system.** Serum-free media and cell homogenate were collected to examine proguanylin secretion as a function of time. Figure 4 depicts a representative Western blot in which the medium was collected at different time points after the serum-free DMEM exchange, along with cell homogenates. Signal from the media began to appear at 4 h and gradually increased as a function of incubation time. A larger amount of proguanylin was present in the basolateral medium than on the apical side of the insert. At no time was there an accumulation of proguanylin within the cell homogenates or the appearance of a plateau of signal as time increased. An RIA-based bioassay that detects increases in intracellular levels of cGMP when cells are incubated with GC-C agonists (5, 13) was performed to detect bioactive peptide in the apical and basolateral spent media as well as the cell homogenates. After 18 h in culture, little signal (0.021–0.028 pg/ml cGMP) was detected by applying spent media from either of the compartments to the bioassay. No signal (0.00 pg/ml cGMP) was detected by applying the cell homogenates to the bioassay. In contrast, STa at $1 \times 10^{-9}$ M generated a response of 0.64 pg/ml cGMP. This suggests that no bioactive peptide was present in C2/bbe1 cells and that very little of the secreted product was active, despite the presence of immunologically recognized proguanylin.

**Modifier effect on proguanylin message and secretion.** Several biologically active compounds, chosen for their ability to affect the GC-C signaling pathway through activation or inactivation, were incubated with C2/bbe1 cells in six-well plates to study the potential feedback regulation of proguanylin expression and secretion (Fig. 5). RNA was collected from the cells, and protein was isolated from the spent media (see MATERIALS AND METHODS). Because each Northern blot was run separately, the Friedman test was used to verify the consistency of the data sets ($Q = 22.83, P \leq 0.0009$). STa, a "superagonist" of GC-C, was incubated at a dose that elicits maximum activation of GC-C. This did not result in a decrease in expression, as would be predicted if guanylin was regulated by negative-feedback

![Fig. 3. Transmission electron microphotograph of C2/bbe1 cells grown on a Transwell insert. At day 14, C2/bbe1 cells formed a single polarized monolayer with an apically directed brush border. Tight-junction complexes can be observed between cells (inset, arrowheads), confirming a morphologically mature monolayer.](http://ajpgi.physiology.org/)

![Fig. 4. Time course of proguanylin secretion in C2/bbe1 cells. Proguanylin signal was detected in the medium at 4 h after incubation with serum-free medium. Signal increased in apical (Ap) and basolateral (Bl) media until a maximum signal was obtained at 24 h, the last time point collected. Proguanylin was also examined in cell homogenates (H) and consistently showed no accumulation in the homogenates relative to the spent media.](http://ajpgi.physiology.org/)

![Fig. 5. Effect of modifiers of the guanylyl cyclase C (GC-C) chloride secretion pathway on proguanylin expression and secretion. Modifiers that activate (heat-stable enterotoxin (STa), IBMX, 8-bromo-cGMP (8-BrcGMP)) or inhibit (glibenclamide) GC-C-mediated chloride secretion were added to media and incubated overnight. Open bars, mean fold change in mRNA expression compared with control as measured by Imagequant software; solid bars, fold change in proguanylin protein detected in spent media as measured by integrated densitometry values. Values are means ± SE. *Significantly different from control ($P \leq 0.05$). Doses were as follows: 500 nM STa ($n = 8$), 250 μM IBMX ($n = 13$), 100 μM 8-BrcGMP ($n = 9$), 100 μM glibenclamide ($n = 8$).](http://ajpgi.physiology.org/)
inhibition. Instead, there was a small but significant increase of proguanylin message (1.7-fold over control, \( P = 0.05 \)) and an increase in protein secretion (1.2-fold over control, \( P = 0.02 \)). In addition, 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor, and 8-BrcGMP, a cell-permeable analog of cGMP, caused minimal increases in proguanylin message and protein secretion, but not to a significant degree. Gibenclamide, an antagonist that blocks guanylin-mediated chloride secretion through inhibition of CFTR, increased proguanylin message 1.5-fold over control (\( P = 0.05 \)). Secreted protein was similarly increased 1.8-fold (\( P = 0.06 \)).

Chloride secretion occurs in intestinal cells via multiple mechanisms distinct from the GC-C pathway. Several mediators that activate or inhibit chloride secretion in a GC-C-independent fashion were incubated with the C2/bbe1 cells to determine their effect on proguanylin expression and secretion (Fig. 6). Cholera toxin, which increases intracellular cAMP, had the largest effect, a 2.8-fold increase in proguanylin message (\( P = 0.02 \)) and a 2.4-fold increase of proguanylin in spent media (\( P = 0.01 \)). Thapsigargin led to a minimal change in expression (1.08-fold, \( P = 0.05 \)) and secretion (1.12-fold, \( P = 0.004 \)). PMA, which initially activates protein kinase C (PKC) and then, after prolonged incubation, depletes cells of PKC, was added at 2 and 24 h. Although the prolonged, 24-h incubation led to an increase in proguanylin mRNA expression (1.7-fold, \( P = 0.01 \)), there was no increase in secreted protein. A 2-h incubation with PMA led to a small increase in mRNA levels (1.15-fold, \( P = 0.08 \)) and protein secretion (1.23-fold, \( P = 0.03 \)).

**DISCUSSION**

Guanylin is an intestinal secretagogue that likely regulates signal transduction pathways that influence chloride and bicarbonate secretion. However, the synthesis and subsequent secretion of guanylin by intestinal cells are incompletely understood. These experiments define an in vitro model in which to study the synthesis and secretion of proguanylin in an intestinal epithelial cell line and serve to further demonstrate the likely possibility of basolateral secretion by human intestinal epithelial cells.

The production of proguanylin message appears to be associated with the differentiation of intestinal cells. In situ hybridization in the rat has demonstrated proguanylin mRNA expression in the upper 20% of the colonic crypts and no signal at the base of the crypts (23). This expression pattern is mirrored in the human colon and the ileum, although signal has also been detected at the base of the crypts in the human small intestine (4). In mouse and human intestinal adenomas (4, 32, 34), a condition in which dedifferentiation is associated with loss of gene expression (8, 39), proguanylin mRNA signal is conspicuously reduced or absent. The association of proguanylin mRNA expression and differentiation appears to be similar in cell lines derived from human intestinal adenocarcinomas. zCaco-2 cells, harvested at various times after seeding, increase mRNA expression by Northern blot as cells reach confluency and differentiate (17). C2/bbe1 cells, a more-differentiated subclone of the Caco-2 phenotype, also show this increase in guanylin message as a function of time after seeding. In addition, we have shown that this more-differentiated cell line demonstrates higher proguanylin mRNA and secreted product than confluent zCaco-2 cells seeded at identical densities and incubated for the same length of time (Fig. 1). This differentiation-dependent increase in guanylin expression does not appear to be an epiphenomenon of the Caco-2 lineage, inasmuch as the N2 and C1 subclones of the HT-29-CP cell line show greater proguanylin message than their less-differentiated parental phenotype (17). The ability of subclones derived from the same original cell line to have different expression patterns of isolated proteins underlies the heterogeneous nature of cells grown in culture. For example, this phenomenon was recently documented in three subclones of Caco-2 cells with regard to the epithelial Na\(^+\)/H\(^+\) exchanger (19). The appearance of proguanylin synthesis and production in C2/bbe1 cells as a function of their level of differentiation makes this a plausible model for the human villous epithelial cell. Furthermore, this cell line, when grown on Transwell inserts, has been useful in the study of vectorial transport. For example, C2/bbe cells have been shown to mimic the apically directed release of secretory leukocyte proteinase inhibitor in the intestine (33).

C2/bbe1 cells secrete an immunoreactive protein into spent media that comigrates with the guanylin fusion protein. The signal is completely inhibited in our blocking studies (Fig. 2). The size of the product and the relative specificity of binding of this product to the Rb 4696-4 antiserum lead us to the conclusion that it represents the proguanylin molecule. When spent medium from C2/bbe1 cells grown on the Transwell inserts was examined, the signal was present in the

![Fig. 6. Effect of modifiers of chloride secretion independent of GC-C on proguanylin expression and secretion. Modifiers that affect chloride secretion independent of the GC-C pathway were added to media and incubated overnight, unless otherwise specified. Open bars, mean fold change in mRNA expression compared with control as measured by Imagequant software; solid bars, fold change in proguanylin protein detected in spent media as measured by integrated densitometry values. Values are means ± SE. *Significantly different from control (\( P \leq 0.05 \)). Doses were as follows: 1 ng/ml cholera toxin (\( n = 9 \)), 2 mM thapsigargin (\( n = 5 \)), 0.1 \( \mu \)M phorbol 12-myristate 13-acetate (PMA, 2-h incubation, \( n = 6 \)), 0.1 \( \mu \)M PMA (24-h incubation, \( n = 6 \)).](http://ajpgi.physiology.org/ by 10.220.33.4 on June 22, 2017)
The bioactive guanylin molecule has been identified in rat colon using a vascularized colonic loop (29) and in Ussing chambers (28). Both models demonstrated a preponderance of apical secretion in the unstimulated colon. Aside from the interspecies disparity between these two experiments and the human-derived C2/bbe1 cells, another major difference may explain the apparent discrepancy between these results and our data. The colonic mucosa consists primarily of goblet cells that have been shown to accumulate guanylin in vivo by immunohistochemistry (24). C2/bbe1 cells are representative of villous-type epithelial cells and may not mimic the secretory patterns of goblet cells. It is plausible that the small amount of immunoreactive protein identified in the rat models represents the contribution of the fewer villous colonocytes in this heterogeneous group of cells.

In the Ussing chamber studies (28), apical and basolateral secretion of bioactive guanylin was increased with the addition of the muscarinic agonist carbachol and 8-BrcGMP for 60 min. The apical secretion of proguanylin was also increased. In the vascularized rat colon model (29), the muscarinic agonist bethanechol mirrored this response. In addition, the neuropeptides bombesin and vasoactive intestinal peptide also stimulated guanylin secretion. Thus there is a relatively rapid response of guanylin to a wide array of secretagogues, suggesting a “pooled” store of guanylin in the rat colon. We wished to determine whether the appearance of immunoreactive peptide in the spent media was a consequence of the accumulation of proguanylin within the cells. As shown in Fig. 4, immunoreactive proguanylin was detected in the apical and basolateral media by 4 h. The signal increased in intensity until the 24-h time point, signifying continuous secretion. The immunoreactive protein was not detected in the cell homogenates, suggesting constitutive secretion, rather than the release of intracellular stores, in this cell culture system. To explain these differences, we speculate that goblet cells, the predominant epithelial cell type in the rat colon, represent a pooled store of proguanylin that can be released into the intestinal lumen and then converted to guanylin. This differentially regulated pattern of secretion would suggest that there may be a distinct pattern of guanylin secretion in ileocytes and colonocytes.

Hormones and secretory peptides are often governed by a negative-feedback loop that directly regulates the control of their release. When the GC-C pathway was inhibited at a distal point by direct blockade of CFTR with glibenclamide, proguanylin appeared to increase in the predicted manner. Several other potential mediators of the GC-C signaling pathway were added to the medium in six-well plates to examine the role of negative-feedback inhibition in the C2/bbe1 cells. STa should cause a downregulation of proguanylin synthesis if this is regulated by feedback inhibition of GC-C stimulation. In fact, the opposite occurred, although the increase in secreted proguanylin was minimal. Cholera toxin, which acts on chloride secretion via cAMP-mediated mechanisms, was the most potent activator of proguanylin expression and secretion. One possible explanation for the increase in proguanylin message by STa is the likely cross activation of protein kinase A by cGMP (35). In addition to cholera toxin, several other agonists that affect secretory pathways were used to probe the regulation of proguanylin secretion. Thapsigargin had little effect on proguanylin production. Similarly, depletion of PKC by incubation with PMA for 24 h or stimulation of PKC by incubation with PMA for 2 h had little effect on proguanylin secretion. PMA incubation for 24 h did, however, increase proguanylin mRNA levels. Further experiments are needed to determine whether the collection of spent media after a more prolonged incubation period would lead to an increase in levels of proguanylin in the media. Carbachol (28) and bethanechol (29) have been used to stimulate guanylin secretion in rat colon preparations. The effects of muscarinic agonists are most likely under vagal control and may not be applicable to cell culture but imply another potential signaling pathway in the intact intestine.

Although the regulatory mechanisms of proguanylin secretion remain to be specifically elucidated, the C2/bbe1 model of proguanylin secretion has led to several observations that may provide clues to the physiological importance of the guanylin molecule. The bidirectional nature of peptide secretion suggests a function more complex than paracrine/autocrine-mediated chloride secretion based on activation of the apical GC-C-activating system (36). A potential site of action for basolaterally secreted proguanylin is the liver. In the rat, GC-C is expressed in the developing liver and after liver injury and repair (20, 21). Furthermore, the presence of an additional receptor that recognizes STa has been suggested in binding assays using IEC-6 cells, a cell line that does not express GC-C (26). A novel receptor for the guanylin-like family of peptides has been found in renal tubular cells in the mouse (1) and opossum (25), suggesting the presence of non-GC-C receptors and a role in fluid homeostasis in the kidney. In addition to its presence in the intestine and its ability to affect renal fluid transport, guanylin has also been shown to be present in the pars tuberalis portion of the pituitary gland, suggesting an extraluminal site of action and a broader endocrine function (9). The existence of a basolateral secretory pathway of proguanylin and the potential for alternative receptors in intestinal and nonintestinal organ systems suggest strongly that there may be a function of proguanylin distinct from GC-C-mediated fluid homeostasis in the intestine. The differences in proguanylin release in rat colon and in the villous-like epithelial C2/bbe1 cell line provide a potential clue to investigate these unique functions, including the regulation of secretion in villous epithelial cells. In contrast to the rat colon studies, in C2/bbe1 cells, proguanylin secretion does not appear to respond to activation of cGMP-mediated pathways.
or calcium-dependent chloride secretion. Proguanylin does appear to be secreted in response to an increase in cAMP, which is similar to the vascularized rat colonic loop that responded to vasoactive intestinal peptide (29). Furthermore, inhibition of CFTR, the ion channel responsible for cGMP- and cAMP-mediated chloride efflux, appears to increase proguanylin expression. Our data support the use of C2/bbe1 cells as a model system in which to further explore the role and mechanisms of guanylin expression and secretion in an in vitro model of intestinal villous cells.

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