Cyclic AMP and Chloride-dependent Regulation of the Apical Constitutive Secretory Pathway in Colonic Epithelial Cells*

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Epithelial cells of the colonic crypt engage in cAMP-mediated fluid and electrolyte secretion. In addition to participating in electrolyte transport, colonic crypt cells also synthesize and secrete a number of proteins and peptides that play a crucial role in mucosal homeostasis. In the present study we show that cAMP regulates not only electrolyte secretion but also polarized protein secretion in a tissue culture model of colonic crypt cells. We found that apical but not basolateral protein secretion was stimulated by a physiological activator of the cAMP pathway, vasoactive intestinal peptide, as well as by a cell-permeant analogue of cAMP (8-(4-chlorophenylthio)cAMP) at concentrations as low as 12.5 μM. Based on several criteria, we determined that the regulation of protein secretion by cAMP in HT29-CL 19A cells occurs via stimulation of constitutive membrane traffic from the trans-Golgi network (TGN) to the apical cell surface. In addition, the regulation of apical protein secretion by cAMP was Cl⁻ dependent with cAMP inhibiting rather than stimulating secretion in Cl⁻ depleted cells. The locus of cAMP action on the secretory pathway is at least in part at the level of the TGN, where it stimulates the sialylation of α 1-antitrypsin (i.e. one of the identified secretory proteins) in addition to the traffic of secretory proteins from the TGN to the apical cell surface. We propose that a cyclic AMP and Cl⁻ dependent regulation of TGN acidification could modulate both sialylation and secretory vesicle budding at the TGN.

The regulated secretory pathway involves the acute, stimulus-induced release of secretory material from a preformed storage compartment, such as the mucin storage granules of goblet cells (7). Conversely, the constitutive secretory pathway lacks well defined storage granules and operates at a considerable basal level of activity. Constitutive membrane traffic pathways are also subject to regulation; e.g. there is accumulating evidence that the rate of constitutive membrane traffic from the Golgi apparatus to the cell surface can be modulated by regulatory factors such as heterotrimeric G proteins and protein kinases (8–12). The molecular mechanisms and physiological stimuli underlying the protein kinase C-dependent regulation of constitutive secretion in rat basophilic leukemia cells have been partially characterized and involve the regulated binding of ARF to Golgi membranes (11). Less is known about the regulatory role of cAMP-dependent protein kinase in constitutive membrane traffic. Apical membrane traffic in Madin-Darby canine kidney epithelial cells is stimulated by cAMP analogs but only at high concentrations (0.5–5 μM 8-Br-cAMP) (10) or in combination with high concentrations of phosphodiesterase inhibitors (500 μM 3-isobutyl-1-methylxanthine) (8). Unresolved issues include: (a) whether or not apical protein secretion can be stimulated by physiological activators of the cAMP-dependent protein kinase pathway (e.g. hormone receptors coupled to adenylyl cyclase) in polarized epithelial cells and (b) the identities of the downstream effectors that mediate the stimulation of constitutive secretion by cAMP-dependent protein kinase.

In an earlier work we demonstrated that cAMP inhibits endocytosis and stimulates the recycling of previously internalized glycoproteins to the cell surface in pancreatic and intestinal epithelial cells that express wild type CFTR (13, 14). Cells that were homozygous for the most common CFTR mutation (AF508) lacked the regulation of either endocytosis or exocytosis by cAMP (14). Because CFTR itself has been shown to enter the recycling pathway (15) and is present in cation-coated vesicles (16), it has been postulated that CFTR modulates the recycling pathway via its function as a cAMP-regulated Cl⁻ channel within this pathway (15). Given that a subset of recycling glycoproteins recycle through the TGN following their endocytosis from the cell surface (17–19), it is conceivable that the recycling and biosynthetic pathways share common compartments and perhaps regulatory elements at the level of the constitutive secretory pathway.

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1 The abbreviations used are: ARF, ADP-ribosylation factor; VIP, vasoactive intestinal peptide; cAMP, 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate; TGN, trans-Golgi network; CFT, cystic fibrosis transmembrane conductance regulator; AT, α 1 antitrypsin; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing; FBS, fetal bovine serum; TEMED, N,N,N′,N′-tetramethylethylenediamine; DMEM, Dulbecco’s modified Eagle’s medium; MEBSS, modified Earle’s buffered salt solution; BAPTA-AM, 1,2-bis(o-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid tetra(aetoxyethyl)-ester.
TGN. On this basis we reasoned that CAMP (perhaps through CFTR) could regulate not only apical membrane trafficking but also protein processing and polarized secretion along the biosynthetic pathway.

In order to assess the role of CAMP in regulating the biosynthetic pathway, we measured polarized protein secretion by colonic epithelial cells (T₈₄ and HT29-CL19A) cultured on permeable supports, characterized the cargo within the apical and basolateral secretory pathways, and determined the effects of CAMP both on polarized secretion and on protein sialylation. We demonstrate that CAMP regulates apical but not basolateral protein secretion by these cells in a dose-dependent manner. The apical secretion by colonic epithelial cells is at least an order of magnitude more sensitive to CAMP analogues than that described for Madin-Darby canine kidney cells and is regulated by a physiological stimulator of the CAMP second messenger system (i.e. VIP). One of the proteins secreted by HT29-CL19A cells was identified as α₁ antitrypsin, which is secreted at both the apical and basolateral surfaces. Based on several criteria, we determined that the secretion of AT from HT29-CL19A cells takes place primarily via the constitutive pathway and that CAMP facilitates the delivery of AT from the TGN to the apical cell surface. In addition, when Cl⁻ was replaced by gluconate, a nonpermeant anion, the regulation was reversed, i.e. CAMP inhibited apical protein secretion. Finally, we observed that cyclic AMP also stimulates the rate of AT sialylation within the biosynthetic pathway, indicating that the TGN is at least one site of action for CAMP. We discuss how CFTR, a CAMP-regulated Cl⁻ channel that has been implicated in the acidification of the TGN and in the regulation of membrane recycling, may be involved in the CAMP-dependent regulation of protein sialylation and apical protein secretion in colonic epithelial cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—Tissue culture media were from Life Technologies, Inc., and defined fetal bovine serum (FBS) was from HyClone (Logan, UT). Acrylamide, bis-acrylamide, ammonium persulfate, TEMED, urea, β-mercaptoethanol, and ampholytes were from Bio-Rad. Protein G immobilized on agarose beads was from Boehringer Mannheim. Dialyzed FBS was prepared by dialyzing FBS against 100 volumes of phosphate-buffered saline overnight at 4°C in dialysis bags (Spectrapor; molecular weight cut-off, 3,500) followed by sterile filtration. Tran-[³⁵S]-label was purchased from ICN (Costa Mesa, CA). Forskolin was from Calbiochem (La Jolla, CA), and Rp-8cpt-cAMP was from Biolog (La Jolla, CA). [³⁵S]-Methionine and [¹⁴C]-Methylmethionine were purchased from ICN (Costa Mesa, CA). Forskolin was from Calbiochem (La Jolla, CA), and Rp-8cpt-cAMP was from Biolog (La Jolla, CA).

**Acrylamide Gel Electrophoresis**—Tissue culture media were from Life Technologies, Inc., and defined fetal bovine serum (FBS) was from HyClone (Logan, UT). Acrylamide, bis-acrylamide, ammonium persulfate, TEMED, urea, β-mercaptoethanol, and ampholytes were from Bio-Rad. Protein G immobilized on agarose beads was from Boehringer Mannheim. Dialyzed FBS was prepared by dialyzing FBS against 100 volumes of phosphate-buffered saline overnight at 4°C in dialysis bags (Spectrapor; molecular weight cut-off, 3,500) followed by sterile filtration. Tran-[³⁵S]-label was purchased from ICN (Costa Mesa, CA). Forskolin was from Calbiochem (La Jolla, CA), and Rp-8cpt-cAMP was from Biolog (La Jolla, CA).

**Macromolecular Assay for the Secretion of Metabolically Labeled Proteins**—For SDS-PAGE analysis, media samples were collected from triplicate cultures and filtered through 0.45 μm-pore-size filters. Aliquots of the filtrates (50 μl) were treated with Proteinase K (20 μg/ml) at 37°C for 1 h. Samples were precipitated with trichloroacetic acid (final concentration 10%), washed in 1% trichloroacetic acid, and dried. Proteins were then dissolved in sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol, and 8% glycerol) and heated to 95°C for 5 min. Gels were electrophoresed in 10% polyacrylamide gel containing 1% SDS and run at 100 V constant for 4 h. Bands were visualized by autoradiography and quantitated by phosphorimager (Fluorchem, Bio-Rad). Images were analyzed using Quantity One software (Bio-Rad). Bands were quantitated by densitometry.

**Immunofluorescence**—Cultures were washed twice with phosphate-buffered saline and fixed in 4% paraformaldehyde for 15 min at room temperature. Cells were then permeabilized with 0.1% Triton X-100 in phosphate-buffered saline for 3 min. After three washes in phosphate-buffered saline, the cultures were incubated with the primary antibody at 1:100 dilution for 60 min at room temperature. After washing with phosphate-buffered saline, the cultures were incubated with FITC-conjugated secondary antibody (1:150 dilution) for 30 min at room temperature. Cells were then washed with phosphate-buffered saline and mounted with 95% glycerol, 5% DAPI, and 0.1% formaldehyde. Images were captured using a confocal microscope (Leica, Wetzlar, Germany). Images were analyzed using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institute of Health, Bethesda, MD, http://imagej.nih.gov/ij/).
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RESULTS

General Considerations Regarding the Approach—In order to assess the regulation of polarized protein secretion by filter-grown cultures of colonic epithelial cells, we analyzed the secretion of metabolically pulse-labeled proteins by three different methods. First, labeled proteins that were secreted into either the apical or basolateral compartment, as well as proteins retained within the cells, were trichloroacetic acid precipitated and quantified by scintillation counting. The rates of secretion were then calculated as the percentage of radiolabeled proteins released into either compartment as a function of time. This technique provided a macroscopic assay of the kinetics and regulation of apical and basolateral secretion. Second, the apical and basolateral media samples were directly analyzed by SDS-PAGE followed by phosphor imaging. This "medium resolution" technique provided an initial qualitative assessment of the cargo within the apical and basolateral secretory pathways and quantitative information regarding the secretion of individual protein bands. Third, we used a combination of two-dimensional gel electrophoresis, immunoprecipitation, and one-dimensional isoelectric focusing to identify specific proteins within the secretory pathway and to monitor protein processing in the biosynthetic pathway.

cAMP Stimulates Apical but Not Basolateral Protein Secretion—Fig. 1 summarizes the results of our initial experiments performed using the macroscopic assay of polarized protein secretion. Both colonic cell lines behaved very similarly regarding the polarized secretion of metabolically labeled proteins analyzed with this method. Secretion exhibited linear kinetics throughout the 120-min chase period and the rate of basolateral secretion by both cell lines was severalfold greater than the apical secretion (Fig. 1, a and b). Secretion in both directions was abolished at 4 °C, as expected for a vesicle-mediated secretory process (Fig. 1b). When the chase was performed in the presence of cpt-cAMP (Fig. 1, a and b), the rate of apical secretion was stimulated significantly over baseline in both colonic cell lines. Forskolin, a direct activator of adenylate cyclase, also stimulated apical secretion by T84 cells (Fig. 1b). Basolateral secretion was unaffected by cyclic AMP in either of these two cell lines. Responsiveness to secretagogues was more consistent for HT29-CL19A cells (Fig. 1, error bars); accordingly, these cells were utilized for the more detailed analysis of the apical secretory pathway (see Figs. 2–7).

Our macroscopic secretion data suggested that protein secretion by colonic epithelial cells is polarized, because the rate of basolateral secretion was severalfold greater than apical secretion. However, this quantitative difference may simply reflect the greater basolateral surface area of these cells as observed by transmission electron microscopy (data not shown) rather than a qualitative difference in the nature of these pathways. In order to assess the profiles of apically and basolaterally secreted proteins and to obtain qualitative information regarding the polarity of secretion, the secretory products were analyzed by SDS-PAGE followed by phosphor imaging and densitometry. This analysis (Fig. 2a) revealed that protein secretion by HT29-CL19A cells is indeed qualitatively polarized. For example, major bands of 105 and 120 kDa were observed exclusively in the basolateral secretion (Fig. 2a, right, arrows), whereas several lower intensity bands were characteristic of the apical pathway (Fig. 2a, left). Interestingly, in spite of the qualitatively distinct protein profiles of the apical and basolateral pathways, many bands were present in both, including two major bands corresponding to 60 and 95 kDa (Fig. 2a, solid arrows). Consistent with our macroscopic data, the apical but not basolateral secretion of the 60 and 95 kDa proteins increased in response to cpt-cAMP, as evidenced by the increased density of the corresponding bands on the phosphor images (Fig. 2a, left). The density of the 60-kDa band that appeared in the apical medium during a 2-h chase period increased by 2.5-fold (i.e. a 150% increase over the density of the apical control band) in the presence of 200 μM cpt-cAMP, as determined by phosphor image analysis (Fig. 2b and Fig. 3a, second column). The stimulation by cpt-cAMP was not restricted to the 60-kDa protein but was generally observed for the majority of apically secreted proteins (Fig. 2a; see 95-kDa band and bands indicated by hollow arrowheads).

Fig. 2b illustrates that the rate of apical secretion was stimulated at cpt-cAMP concentrations as low as 12.5 μM. Shown is the dose-response relationship between the concentration of cpt-cAMP and the relative stimulation of secretion of the 60-kDa band (Fig. 2b). We observed a characteristic two-phase regulation of apical secretion by cAMP. In the first phase, concentrations of cpt-cAMP between 0–100 μM caused a 2-fold stimulation of secretion, plateauing at 50 μM (Fig. 2c). In the second phase, concentrations of cpt-cAMP greater than 100 μM resulted in additional stimulation of secretion that plateaued at approximately 500 μM cpt-cAMP and that corresponded to a nearly 4-fold stimulation over control values.

In order to document that cpt-cAMP stimulated apical protein secretion by a cAMP-specific mechanism, we utilized a panel of pharmacological mediators that act on the cAMP signaling pathway in different ways. Fig. 3 summarizes our data regarding the pharmacological profile of regulation, as assessed by phosphor imaging and densitometry of the 60-kDa protein. 10 μM forskolin added to both sides or 100 nM VIP added to the basolateral side (i.e. to the side where its receptors

![Fig. 1. Regulation of polarized protein secretion as determined by trichloroacetic acid precipitation and scintillation counting.](https://example.com/fig1.png)

The figure shows the results of the macroscopic assay of polarized protein secretion. The graphs illustrate the secretion rates in both apical and basolateral compartments for HT29-CL19A and T84 cells. The secretion rates are shown as a percentage of radiolabeled proteins released over time. The data are presented in two panels, labeled a and b, representing different cell lines: HT29-CL19A and T84 cells, respectively. The graphs demonstrate that the rate of basolateral secretion is severalfold greater than the apical secretion, and that this secretion is inhibited at 4 °C. The addition of cpt-cAMP significantly stimulates apical secretion, as indicated by increased radioactivity in the apical compartment.
are present) evoked increases in apical secretion similar in magnitude to that induced by 200 μM cpt-cAMP (see Fig. 3a). VIP added to the apical side had no effect, and the administration of 500 μM Rp-8-cpt-cAMPS, a cAMP-antagonist, abolished the effect of basolaterally administered VIP. The same treatments had no significant effects on the basolateral secretion of the 60-kDa protein (Fig. 3b). Given that the only common feature of these three agents is their ability to activate the cAMP signaling pathway, we conclude that cAMP is a specific regulator of apical protein secretion by HT29-CL19A cells.

Identification of the 60-kDa Secretory Protein as α1-Antitrypsin—In order to begin identifying some of the proteins secreted by HT29-CL19A cells, we analyzed the secreted proteins by two-dimensional PAGE and compared the results with the SWISS two-dimensional PAGE database (27). The 60-kDa secreted protein was identified as AT, based on the characteristic appearance of its multiple sialylated forms, its isoelectric point, and its molecular weight (Fig. 4, arrow). This finding was verified by immunoprecipitation using specific antisera and relevant controls (data not shown). Two-dimensional PAGE analysis that was performed on both apically and basolaterally secreted proteins confirmed that AT as well as the 95-kDa protein (Fig. 4, asterisk) is secreted bidirectionally (only apically secreted proteins are shown). We also compared the apical secretions with or without stimulation by 200 μM cpt-cAMP by two-dimensional PAGE and failed to observe any novel protein species that were released by the cells as a consequence of cAMP stimulation (data not shown).

Apical Protein Secretion Exhibits Linear Kinetics and Is Ca2+-Insensitive—In order to further characterize the apical secretory pathway that is regulated by cAMP in HT29-CL19A cells, we evaluated the time-course of secretion over an extended period of time and the Ca2+ dependence of polarized protein secretion. Fig. 5 (a and b) illustrates that AT secretion by HT29-CL19A cells follows a linear time course over 6 h in both the apical (Fig. 5a) and the basolateral (Fig. 5b) directions. In the presence of 200 μM cpt-cAMP, the basolateral rate of secretion was unchanged, whereas the same cpt-cAMP concentration elicited a sustained increase in the rate of apical AT secretion. Fig. 5c demonstrates that the Ca2+ ionophore, iono-
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FIG. 4. Two-dimensional PAGE analysis of apically secreted proteins; α1-antitrypsin as the predominant secretory protein. The direction of the first dimension is indicated. Molecular markers mass were run in parallel (left side of panel, 200, 97.4, 69, 46, and 30 kDa). The arrow indicates the protein that corresponds to the 60-kDa protein observed on one-dimensional gels. Isoelectric point of this protein corresponds to 4.9–5.3 as determined by comparison with two-dimensional protein markers (Bio-Rad). The position and appearance of this protein is identical to the position and appearance of α1-antitrypsin secreted by HepG2 cells (Swiss 2D databank). The protein corresponding to the 95-kDa band (asterisk) has not been identified.

FIG. 5. Linear kinetics and Ca\(^{2+}\) independence of AT secretion in the absence or the presence of cAMP. a and b, representative data from one experiment are shown. Individual data points represent the intensity of the 60-kDa protein band determined by phosphor image analysis of apical (a) and basolateral (b) samples collected following various chase periods in the presence or the absence of cTP-cAMP (200 μM). Correlation coefficients from linear curve fitting: apical control R = 0.976, apical cAMP R = 0.979, basolateral control R = 0.965, basolateral cAMP R = 0.945. c, extracellular [Ca\(^{2+}\)] and ionomycin (1 μM) have no effect on AT secretion. Shown are the normalized mean intensities of the 60-kDa apical band following 2 h of chase as determined by phosphor image analysis of duplicate samples from two experiments (mean ± S.E., n = 4). Individual data points were normalized to the mean intensity of the 60-kDa apical band determined for duplicate samples under control conditions. The presence (+) or the absence (−) of extracellular Ca\(^{2+}\) (1 mM) during the chase is indicated.

Cyclic AMP-induced Cl\(^{-}\) secretion by these cells (data not shown); thus, the inability of ionomycin to stimulate protein secretion in these cells is not due to a lack of effect on intracellular Ca\(^{2+}\) activity. Neither ionomycin (data not shown) nor the removal of extracellular Ca\(^{2+}\) (Fig. 5c) affected the extent of stimulation by cAMP. Furthermore, incubation of the monolayers with 20 μM BAPTA-AM for 60 min prior to metabolic labeling and during the subsequent labeling and chase periods did not affect either the polarity or the cAMP-dependent regulation of secretion (data not shown). Thus, apical protein secretion by HT29-CL19A cells and its regulation by cAMP are largely insensitive to intracellular Ca\(^{2+}\) ions.

Constitutive Apical Secretion in HT29-CL19A Cells Is Regulated by cAMP in a Cl\(^{-}\)-dependent Fashion—Because CFTR (i.e., a CAMP-regulated Cl\(^{-}\) channel) has been implicated in the regulation of apical membrane traffic (see the introduction), we also examined the Cl\(^{-}\) dependence of the effect of cAMP on apical protein secretion. Fig. 6 demonstrates the results of a representative experiment in which the secretion assay was performed either in tissue culture medium (Fig. 6, white bars), a buffered salt solution containing Cl\(^{-}\) (Fig. 6, black bars), or a physiological buffer solution instead of tissue culture medium (Fig. 6, light bars). However, when the Cl\(^{-}\) was replaced by gluconate (Fig. 6, hatched bars), i.e., an anion that is poorly conducted by most Cl\(^{-}\) channels (28–31). A stimulation of apical secretion by cAMP was also observed when the assay was performed in a physiological buffer solution instead of tissue culture medium (Fig. 6, black bars). However, when the Cl\(^{-}\) in this buffer was replaced by gluconate, cAMP failed to stimulate secretion. Instead, the cAMP inhibited apical protein secretion in Cl\(^{-}\)-depleted cells (Fig. 6, hatched bars).

Cyclic AMP Also Stimulates Protein Sialylation: Regulation at the TGN—Inhibition of TGN acidification results in reduced protein sialylation and in a reduced rate of constitutive protein secretion in HepG2 cells (32). Because CFTR, a CAMP-regulated Cl\(^{-}\) channel, has been implicated in TGN acidification in those cells that express it (33–36), we reasoned that the regulation of constitutive secretion by cAMP in colonic epithelial cells could be related to a cAMP-dependent regulation of TGN.
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**DISCUSSION**

**cAMP Regulates Constitutive Membrane Traffic from the TGN to the Apical Cell Surface**—Our results indicate that cAMP regulates apical but not basolateral protein secretion in colonic epithelial cells. The specificity of this regulation by cAMP was documented using a panel of secretagogues including a physiological activator of the cAMP pathway (i.e., VIP), the stimulatory effect of which was blocked by a stereo-specific inhibitor of cAMP. The apical secretory pathway in colonic epithelial cells is at least an order of magnitude more sensitive to cAMP analogs than that reported for Madin-Darby canine kidney cells (8, 10). It remains to be determined if this differential sensitivity of the biosynthetic pathway to cAMP is due to differences in cAMP metabolism or signaling between these cell types (e.g., differences in the expression levels of phospholipase C or cAMP-dependent protein kinase isoforms) or due to different downstream effector mechanisms.

On the basis of the following considerations we conclude that cyclic AMP regulates protein secretion by stimulating constitutive membrane traffic to the apical cell surface: (a) the linear kinetics of secretion under both control and stimulated conditions, (b) the insensitivity of secretion to Ca<sup>2+</sup>, a classical stimulator of regulated secretory pathways, and (c) the lack of accumulation of fully processed secretory material in well-defined storage granules in these cells (data not shown). The observed stimulation of apical protein secretion by cAMP is not due to an elevated rate of protein synthesis, because that would have affected the basolateral rate of secretion. In addition, we determined that there is no further incorporation of radiolabeled amino acids into trichloroacetic acid precipitable material following the initial pulse period (i.e., during the time period when secretagogues are present; data not shown). The polarity of regulation indicates that TGN to apical cell surface traffic is at least one site of regulation by cAMP (i.e., there are no known polarized compartments within the biosynthetic pathway proximal to the TGN). This notion is also in agreement with the finding that the regulatory subunit (R1I) of cAMP-dependent protein kinase associates with the TGN in epithelial cells (38). A feasible mechanism by which cAMP could regulate constitutive membrane traffic is the stimulation of secretory vesicle formation at the TGN, similar to the regulation of constitutive secretion in rat basophilic leukemia cells by protein kinase C (11). In rat basophilic leukemia cells the regulation of constitutive secretion by protein kinase C involves a stimulation of ARF binding to Golgi membranes that drives coat formation and vesicle budding from the Golgi (39). AT secretion in HT29-CL19A cells is likely ARF-dependent, because we observed that brefeldin A (i.e., a drug that inhibits the exchange of guanine nucleotides bound to ARF (40)) completely blocks protein secretion by HT29-CL19A cells (data not shown). Therefore, it is conceivable that mechanisms that regulate ARF binding to the TGN in a cAMP-dependent manner could modulate constitutive secretion in HT29-CL19A cells (see below).

**TGN Acidification as a Possible Mechanism Underlying the Regulation of Constitutive Secretion by Cyclic AMP**—Both the rates of constitutive secretion and of AT sialylation have been shown to be dependent on TGN acidification in HepG2 cells. Namely, concanamycin B, an inhibitor of vorticolar ATPases, decreased the rate of AT secretion and sialylation in these cells (32). The dependence of sialylation on TGN acidification is probably due in part to the acidic pH optimum of sialic acid transferase (41); however, the molecular mechanisms that link acidification to secretion are unknown. Interestingly, ARF binding to microsomal membranes in vitro has been shown to be dependent on vesicular acidification (42). The acidification dependence of ARF binding to membranes could account for the inhibitory effect of concanamycin B on the rate of constitutive secretion in HepG2 cells and provides a feasible mechanism by which cAMP could regulate secretion (i.e., by regulating TGN acidification) in HT29-CL19A cells. Our observation of a stimulatory effect of cAMP on the rate of sialylation of AT that was entrapped in the TGN using a 20°C temperature block is consistent with the notion that cAMP enhances TGN acidification in HT29-CL19A cells (see the connection between sialylation and acidification above). Vesicular acidification requires a mechanism to shunt the membrane potential generated by the vorticolar H<sup>+</sup>-ATPase that otherwise limits the accumulation of protons. A Cl<sup>-</sup> conductance of vesicular membranes can function as such a shunt mechanism, and a cAMP-regulated Cl<sup>-</sup>...
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...protein secretion by cAMP may have a common origin, propose that the regulation of protein sialylation and apical secretory product of HT29-CL19A cells as by cAMP in colonic crypt cells. We identified the predominant and electrolyte and fluid secretion are regulated coordinately of a high sensitivity, cAMP-dependent regulation of apical secretory vesicles of a high sensitivity, cAMP-dependent regulation of apical secretory vesicles. It remains to be determined if budding which has been shown to participate selectively in the apical direction-specific cytosolic factors or both are responsible for polarity of regulation of the constitutive secretory pathway by cyclic AMP. Concluding Remarks—Our data demonstrate the existence of a high sensitivity, cAMP-dependent regulation of apical secretion by colonic epithelial cells. Thus, apical protein secretion and electrolyte and fluid secretion are regulated coordinately by cAMP in colonic crypt cells. We identified the predominant secretory product of HT29-CL19A cells as α1-antitrypsin and provided compelling evidence that the regulation of protein secretion by cAMP in these cells represents the stimulation of constitutive membrane traffic from the TGN to the apical cell surface. Our data are consistent with the notion that protein secretion via the constitutive secretory pathway takes place via the stimulation of constitutive vesicle generation (i.e. budding) from the TGN, in contrast to the regulation of secretory granule consumption (i.e. regulated exocytosis) by glandular cells. We propose that the regulation of protein sialylation and apical protein secretion by cAMP may have a common origin, i.e. regulated TGN acidification. This hypothesis is consistent with the genetic evidence that cyclic AMP-regulated CFTR Cl- channels regulate protein sialylation and TGN acidification in epithelial tissues and is supported by the Cl- dependence of the regulation of protein secretion. Defining the molecular basis for the regulation of protein processing and secretion by cyclic AMP, including the possible role of CFTR in this process, should contribute to our understanding of how polarized epithelial cells modulate mucosal homeostasis.

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