Synthesis of mucous glycoproteins by rabbit tracheal cells *in vitro*

Modulation by substratum, retinoids and cyclic AMP

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One function of airway epithelium is the secretion of mucins, which comprise an important component of the mucous lining layer. We demonstrate that rabbit tracheal epithelial cells grown in primary culture incorporate [³H]glucosamine into material released into the medium which is characterized as mucin by the following criteria: (a) high M_r , (b) monosaccharide composition, (c) ion-exchange behaviour different from that of glycosaminoglycans and (d) oligosaccharides attached via *N*-acetylgalactosamine. The production of mucin by the cells requires growth on a substratum of collagen gel and is enhanced by retinoids in the extracellular medium. In the presence of retinoids, 8-bromo cyclic AMP and factors present in medium from 3T3 fibroblasts each further stimulate mucin production. These results indicate that an isolated epithelial-cell culture system, in the absence of nervous, mesenchymal or other tissue types, can be used to answer questions about the regulation of mucin production at the cellular level.

INTRODUCTION

Normal airway epithelium is composed of several cell types, each of which contributes to the functional aspects of the tissue. Among secretory cells, surface goblet cells and submucosal gland cells vary in relative amount from species to species and with airway level (Plopper *et al.*, 1983) and each makes distinctive contributions to the composition of the mucous layer (Sherman *et al.*, 1981). The high- M_r glycoproteins or mucins secreted by these cells confer viscosity upon their solutions and thus play an important role in mucociliary transport and clearance. The regulation of secretion of these molecules has been studied in whole animals, in organ culture and, most recently, in cell culture, by methods which include both ultrastructural analysis of the cell types present and quantitative and qualitative biochemical analysis of the secretory products. For the study of the regulation of differentiation, primary cell culture has a distinct advantage over organ-culture or whole-animal systems, since it offers a proliferating cell population which can be subjected to manipulation designed to enhance or inhibit the differentiation process. We have adapted previously established procedures (Wu & Smith, 1982; Lee *et al.*, 1984) in order to grow rabbit tracheal epithelial (RbTE) cells under conditions where mucins are produced *in vitro*. The present paper describes the characterization of these molecules which serve as indicators of mucous differentiation. The production of mucins appears further to be regulated both by the substratum upon which the cells are grown and by components of the extracellular medium.

EXPERIMENTAL PROCEDURES

Materials

The retinoid, (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)propen-1-yl]benzoic acid

was provided by Dr. Peter Sorter of Hoffmann-La Roche (Nutley, NJ, U.S.A.). The synthesis and characterization of this compound have been reported (Loeliger *et al.*, 1980). All other materials were obtained from commercial sources.

Cell culture

Tracheal epithelial cells from New Zealand White rabbits (Hazleton Research Laboratories, Denver, PA, U.S.A.) were isolated by proteinase digestion and cultured on FAV-coated dishes as described by Jetten & Smits (1985). The culture medium consisted of Ham's F-12 containing insulin (10 µg/ml), transferrin (5 µg/ml), epidermal growth factor (25 ng/ml), bovine hypothalamic extract (0.1%), penicillin (100 units/ml), streptomycin (100 µg/ml) and gentamycin (50 µg/ml). Cells were plated at 5×10^4 /60 mm-diameter dish and incubated at 37 °C in a humidified atmosphere containing air/CO₂ (19:1). The medium was changed on day 1, 4, 6, 8 etc. after plating. For growth on collagen gels (Yang *et al.*, 1981), cells were subcultured from FAV-coated dishes after 5 or 6 days of growth by dissociation with trypsin/Na₄EDTA (Gibco Laboratories, Grand Island, NY, U.S.A.) at concentrations of 0.1 and 0.04 g/l respectively. After 5 min at 25 °C, an equimolar amount of soybean trypsin inhibitor was added and the cells were washed twice with PBS and plated at 2×10^5 cells per 35 mm-diameter dish on collagen gels with the same medium and growth conditions described above. Medium was changed on day 2, 4, 6 etc. after plating. Dishes were incubated for 48 h with one of the following radiolabelled compounds (per ml of culture medium): 10 µCi of [6-³H]glucosamine, 10 µCi of [6-³H]galactose or 25 µCi of [2-³H]mannose. After collection, the radio-labelled medium was centrifuged at 1000 g for 10 min at room temperature to remove cells and debris, then freeze-dried and redissolved in PBS containing 5% (v/v) 2-mercaptoethanol and 5% (w/v) Chaps.

Abbreviations used: FAV, fibronectin/albumin/Vitrogen; PBS, Dulbecco's (1954) phosphate-buffered saline; Chaps, 3-[(3-cholamidopropyl)dimethylammonio]propanesulphonic acid; RbTE, rabbit tracheal epithelial.

Enzymic digestions

Portions of high- M_r cell secretions were digested with enzymes under the following conditions: hyaluronidase (Miles) from *Streptomyces* (10 units/ml) at 37 °C for 16 h in PBS adjusted to pH 6.0 by the addition of 0.1 M-citric acid; neuraminidase from *Vibrio cholerae* (Calbiochem), 16 munits/ml at 37 °C for 4 h in 0.05 M-sodium acetate/0.15 M-NaCl/0.01 M-CaCl₂, pH 5.0.

Chemical digestion

Portions of high- M_r cell secretions were subjected to the following chemical treatments. Reductive β -elimination was performed in 50 mM-NaOH/1 M-NaBH₄ at 37 °C for 16 h. After neutralization with acetic acid, borate was removed by repeated evaporation with methanol containing 1% acetic acid. Hydrolysis by strong acid was performed in 2 M-HCl for 4 h at 100 °C. After evaporation to dryness, samples were re-*N*-acetylated and desalted as described by Takasaki & Kobata (1978).

Chromatography

Gel-filtration chromatography was performed on 0.7 cm $\times$ 45 cm columns, and 0.36 ml fractions were collected [except chromatography on Bio-Gel P-6DG (1 cm $\times$ 19 cm column, 0.6 ml fractions)]. The eluting buffer was PBS containing 5 mM-dithiothreitol/0.5% Chaps/0.02% NaN₃, except for Bio-Gel P-6DG columns, which were eluted with 0.1 M-NH₄HCO₃. Ion-exchange chromatography was performed on a column (1.0 cm $\times$ 2.5 cm) of DEAE-Sephacel (Nakazawa *et al.*, 1983). The sample was applied in 8 M-urea/0.15 M-NaCl/0.05 M-sodium acetate, pH 6.0, containing 0.5% (w/v) Chaps. After washing the column with 8 ml of this buffer, elution was continued by applying a gradient of NaCl from 0.15 M to 1.4 M over 46 ml. Fractions (0.8 ml each) were collected at a flow rate of 3.0 ml/h. Descending paper chromatography was performed on Whatman no. 1 paper, the solvent being butan-1-ol/pyridine/water (6:4:3, by vol.). The paper was first borate-impregnated by dipping in 0.57% Na₂B₄O₇·10 H₂O/10 mM-NaCl and drying before the sample was spotted.

RESULTS

Primary rabbit tracheal epithelial cells cultured on a substratum of FAV exhibit a stratified squamous morphology, reminiscent of a keratinizing epithelium, and give rise *in vitro* to cross-linked envelopes. The addition of retinoids to the culture medium inhibits cross-linked-envelope formation along with several other markers of squamous differentiation (Jetten & Smits, 1985). The same cells grown on collagen gels not only yield cells with a squamous morphology in the absence of retinoids, but also upon retinoid treatment give rise to areas of polarized cells which contain densely staining granules near the cell apices of the uppermost layer of cells. An electron micrograph of cells grown in the presence of retinoids on collagen gel is shown in Fig. 1. Although addition of retinoids to cells grown on FAV results in the inhibition of cross-linked-envelope formation (Jetten & Smits, 1985), no granular cells were ever detected. As a more quantitative measure of the expression of secretory characteristics by tracheal-cell cultures, the incorporation of radioactive precursors into

glycoconjugates secreted into the culture medium was measured. Cells grown on either substratum, with or without retinoids, secreted material which had incorporated [³H]glucosamine and was eluted in the excluded volume on Sepharose CL-4B chromatography (Figs. 2a and 2b). Upon treatment with hyaluronidase, all of the high- M_r material synthesized by cells grown on FAV was digested, indicating that it was composed exclusively of hyaluronic acid (Fig. 2c). Cells grown on collagen gel in the presence of retinoids consistently gave rise to a high- M_r component resistant to hyaluronidase digestion (Fig. 2d). Preparative separation of this component from hyaluronic acid was accomplished by ion-exchange chromatography, as shown in Fig. 3. On this column, proteins and glycoproteins emerge unretarded or very early in the NaCl gradient, whereas hyaluronic acid is eluted at NaCl concentrations between 0.2 and 0.25 M (Nakazawa *et al.*, 1983). No other component was co-eluted with hyaluronic acid in Fig. 3, since the pooled material was completely susceptible to hyaluronidase digestion (results not shown). Sulphated glycosaminoglycans, if present, would be eluted between 0.25 M- and 0.6 M-NaCl (Chang *et al.*, 1983; Nakazawa *et al.*, 1983). Negligible radioactivity was detected in this region, effectively ruling out this class of compound as a possible component of the high- M_r secretory material.

The RbTE cells in culture secreted insufficient quantities of material for the application of classical techniques to determine sugar and protein compositions. Therefore, to investigate the monosaccharide composition of the putative high- M_r glycoproteins obtained from the unretarded fraction of DEAE-Sephacel chromatography, cells were metabolically radiolabelled with other precursors in addition to [³H]glucosamine. The high- M_r glycoprotein fraction was obtained as described above, that is, by DEAE-Sephacel chromatography of the Sepharose CL-4B-excluded material. Radioactive material eluted unretarded by the DEAE-Sephacel column was dialysed against 0.02% NaN₃, dried by vortex-mixing under reduced pressure and hydrolysed with acid as described in the Experimental procedures section. The results of paper chromatography of the acid hydrolysates are shown in Fig. 4. Radioactivity from [³H]glucosamine is incorporated into both *N*-acetylglucosamine and *N*-acetylgalactosamine (Fig. 4a). A third monosaccharide which might be expected to be labelled by [³H]glucosamine is sialic acid (Yurchenco *et al.*, 1978). The presence of sialic acid in the high- M_r glycoproteins was confirmed by its susceptibility to neuraminidase (Fig. 5), since a quarter of the radioactivity can be released as sialic acid by neuraminidase digestion. Radioactivity from [³H]galactose appears exclusively in the high- M_r material as galactose (results not shown), whereas the incorporation of radioactivity derived from [³H]mannose was negligible.

To determine the sugar involved in the glycosidic linkage with the peptide chain, the high- M_r glycoproteins labelled with [³H]glucosamine were subjected to reductive β -elimination as described in the Experimental procedures section. This treatment resulted in a quantitative release of radiolabelled material, presumably as reduced oligosaccharides, which were eluted in the included volume on Bio-Gel P-10 chromatography (results not shown). This β -eliminated material, when treated with strong acid, gave rise to a new ³H-containing peak on paper chromatography which co-migrated with standard

Fig. 1. Transmission electron micrographs of rabbit tracheal epithelial cells grown on either collagen gel (a) or FAV-coated plastic (b)

The positions of the collagen gel and plastic are indicated by the letters C and P respectively. The arrows indicate densely staining granules reminiscent of secretory granules.

[^{14}C]N-acetylgalactosaminitol (Fig. 4b). No ^3H -containing material co-migrated with N-acetylglucosaminitol, indicating that N-acetylgalactosamine was the sole linkage sugar.

In summary, the results presented above support the conclusion that the high- M_r material unretarded by a DEAE-Sephacel column is composed of mucous glycoproteins; this is based on the following characteristics: (a) high M_r ; (b) ion-exchange behaviour different from that of glycosaminoglycans; (c) monosaccharide composition (GlcNAc, GalNAc, sialic acid, galactose, but no

mannose); and (d) oligosaccharides are attached to polypeptide via a mild-alkali-labile linkage involving N-acetylgalactosamine.

With evidence in hand that RbTE cells in culture secrete mucous glycoproteins, we decided to determine whether the production of this type of molecule could be modulated by changing the components included in the extracellular medium. To this end we grew cells on collagen gel in the presence of various concentrations of the synthetic retinoid or arotinoid (*E*)-4-[2-(5,6,7,8-tetramethylnaphthalen-2-yl)propen-1-yl]benzoic acid. As

Fig. 2. Elution profiles of gel-filtration chromatography on Sepharose CL-4B

Medium from either [$6\text{-}^3\text{H}$]glucosamine [^{35}S]sulphate-labelled cells grown on FAV-coated dishes (a) or [$6\text{-}^3\text{H}$]glucosamine-labelled cells grown on collagen gels (b) was subjected to chromatography as described in the Experimental procedures section. High- M_r material eluted in the void volume was pooled, digested with *Streptomyces* hyaluronidase and the digests applied to columns of Sepharose CL-4B; (c) hyaluronidase digest of high- M_r material from cells grown on FAV-coated dishes; (d) hyaluronidase digest of high- M_r material from cells grown on collagen gel. The arrows indicate the elution positions of Blue Dextran (V_0) and NaCl (V_t). The symbols indicate ^3H (●) and ^{35}S (○) radioactivity. The broken lines indicate changes in scale: (a) $10^{-5} \times \text{d.p.m.}$; (b) $10^{-4} \times \text{d.p.m.}$

mentioned above, cells grown in the absence of retinoids contain very few densely staining granules, which may be involved in mucous secretory activity. The addition of the arotinoid not only stimulates the appearance of granules (Fig. 1), but also enhances the amount of [^3H]glucosamine which is incorporated into high- M_r material, unretarded by a DEAE-Sephacel column (Fig. 6). The effect of the arotinoid is half-maximal at $2 \times 10^{-11} \text{ M}$, which is the same half-maximal concentration for its effect on other differentiation phenomena (Sporn & Roberts, 1984). Retinoic acid likewise enhances mucin production in a concentration-dependent manner, with a half-maximal effective concentration at $5 \times 10^{-10} \text{ M}$ (results not shown), which is a somewhat higher concentration than that required for a half-maximal effect in other systems (Dawson *et al.*, 1985).

In addition to retinoids, several other classes of compounds which have been shown to induce differentiation in other systems (Leder & Leder, 1975; Reuben *et al.*, 1976; Jones & Taylor, 1980; Newton *et al.*, 1980) were tested for their effect on mucin production (Table 1). When added in the presence of an optimal concentration of retinoids, 1 mM-sodium butyrate, 2.5 μM -5-azacytidine

and 1 mM-hexamethylenebisacetamide were ineffective in enhancing [^3H]glucosamine incorporation into mucins. By contrast, the inclusion of 0.5 mM-8-bromo cyclic AMP increased mucin production to 2.7 times that of the standard medium including retinoids. Addition of 8-bromo cyclic AMP in the absence of retinoids was without effect. Mucin production was also enhanced by culturing the cells in a 1:1 mixture of the usual medium and medium harvested from 3T3 fibroblasts ('conditioned medium'). The addition of conditioned medium and arotinoid together resulted in a dramatic 35-fold increase in mucin production over that of cells grown in the absence of conditioned medium and arotinoid. Addition of 8-bromo cyclic AMP resulted in a slight further increase.

DISCUSSION

The results presented demonstrate that RbTE cells *in vitro* can produce mucins and that this production is affected both by the substratum upon which the cells are grown and by factors present in the extracellular medium. Production of mucin by airway cells in culture has been demonstrated in two other species: pig (DeBuysscher *et al.*, 1984) and hamster (Kim *et al.*,

Fig. 3. Anion-exchange chromatography of [^3H]glucosamine-labelled high- M_r material from RbTE cells grown on collagen gels

Pooled radioactive medium from ten dishes was subjected to chromatography on Sepharose CL-4B as described in the Experimental procedures section. Radioactive material eluted in the void volume was made 8 M in urea and 0.5% (w/v) in Chaps and applied to a column of DEAE-Sephacel, which was eluted as described in the Experimental procedures section. Radioactive material eluted unretarded from the column (fractions 4–17) was pooled for further analysis. The arrows indicate: 1, application of the sample; 2, elution with equilibration buffer; 3, elution with the NaCl gradient. The continuous line indicates radioactivity, whereas the broken line indicates NaCl concentration as deduced from conductivity measurements.

1985). In addition, Kim (1985) has recently reported results from the rabbit which are consistent with our own. In each case, a collagen substratum was used which ultimately resulted in a culture producing mucin. DeBuysscher *et al.* (1984) did not systematically evaluate the effect of collagen substratum on mucin production, except to observe that using dishes coated with collagen resulted in an increased attachment of cells with an epithelial morphology. Tracheal cells from the hamster grow poorly on either uncoated or collagen-coated dishes (Lee *et al.*, 1984). A collagen gel seems to be required for optimal proliferation. By contrast, rabbit tracheal cells can proliferate rapidly both on collagen gels and on other types of substrata (Wu *et al.*, 1982), thus facilitating the comparison between substrata shown in Fig. 2. The results indicate that collagen gel is required for mucin production (see also Kim, 1985), by analogy to the mammary epithelium, where culture on collagen gels has a strong positive influence on morphology and secretory function (Burwen & Pitelka, 1980). In both the tracheal and the mammary cells, therefore, the extracellular matrix and growth on collagen gel in particular dispose the cells toward expression of specific differentiated functions or enhance the process of differentiation itself. In the RbTE system, growth on FAV in the absence of retinoids seems to lead to expression of cellular products which are consistent with squamous differentiation (Jetten & Smits, 1985). The substratum may be one factor which modulates differentiation *in vitro* along the normal pathway of mucosecretory differentiation.

Another factor known to influence the development of the squamous phenotype is availability of retinoids.

Fig. 4. Paper chromatography of strong-acid hydrolysates of RbTE-cell [^3H]glucosamine-labelled high- M_r material unretarded on ion-exchange chromatography before (a) and after (b) β -elimination

^{14}C -labelled internal standard *N*-acetylhexosamines and *N*-acetylhexosaminotols were included before the acid hydrolysis to monitor recoveries and unambiguously determine migration positions. The arrows indicate the migration of the standards: 1, *N*-acetylglucosaminitol; 2, *N*-acetylgalactosaminitol; 3, *N*-acetylgalactosamine; 4, *N*-acetylglucosamine. ●, ^3H derived from the cellular material; ○, ^{14}C radioactivity derived from the internal standards.

Vitamin A-deficient animals develop keratinized tracheas, and the inhibition of this phenomenon has served as the basis of a bioassay for retinoids (Sporn & Roberts, 1984). Likewise, *in vitro*, retinoids can prevent squamous differentiation by RbTE cells grown on FAV (Jetten & Smits, 1985). Given the additional stimulus of the collagen substratum, retinoids induce the production of mucins in a concentration-dependent manner (Fig. 6). Taken together with the results of Fig. 2, retinoids can be termed a necessary, but not sufficient, factor for mucin production, since, even in the presence of retinoids, cells grown on FAV produce no mucin. Whether retinoids have other effects on mucin biochemistry, such as influencing oligosaccharide chain length or composition, remains to be determined. Although the molecular mechanism of action of retinoids is currently a subject of controversy, some details are becoming clear, especially in the RbTE system. Specifically, when cells are grown on FAV, retinoids reduce the amount of mRNA present for two markers of squamous differentiation, namely that of transglutaminase I and of a 48 kDa keratin. Thus the regulation of the amounts of these markers by retinoids may occur at a transcriptional level, and the target site of retinoid action is the chromatin. If retinoids are to act

Fig. 5. Gel-filtration chromatography of a neuraminidase digest of [3 H]glucosamine-labelled RbTE-cell high- M_r material unretarded on ion-exchange chromatography

A portion of unretarded material (see Fig. 3) was subjected to neuraminidase digestion and chromatography on a column of Bio-Gel P-6 as detailed in the Experimental procedures section. The arrows indicate: V_0 , the elution position of bovine serum albumin; V_{sia} , the elution position of an authentic free *N*-acetylneuraminic acid standard; and V_t , the elution position of NaCl.

Table 1. Effect of various medium components on mucin production by RbTE cells *in vitro*

Cells were plated on collagen gels as described in the Experimental procedures section and the following were added on the indicated days after plating: 10^{-10} M-arotinoid (+AR) and/or conditioned medium, days 3–9; 1 mM-sodium butyrate/2.5 μ M-5-azacytidine/1 mM-hexamethylenbisacetamide (+HMBA)/0.5 mM-8-bromo cyclic AMP, days 6–7. Dishes were incubated with [3 H]glucosamine for 48 h from day 7 to day 9. Mucin production was determined as described in the legend to Fig. 6.

Conditions	Mucin production (d.p.m./ 10^3 cells)
Expt 1.	
No additions	1.5
+AR	20.2
+AR + sodium butyrate	19.5
+AR + 5-azacytidine	16.7
+AR + HMBA	20.3
+AR + 8-bromo cyclic AMP	55.5
+8-bromo cyclic AMP	1.4
Expt. 2.	
No additions	1.9
+ Conditioned medium	9.5
+ AR + conditioned medium	66.6
+ AR + conditioned medium + 8-bromo cyclic AMP	82.0

transcriptionally to regulate mucin production, then, in contrast with the squamous markers, which are decreased, retinoids must have a positive effect on gene expression as well as the negative effects already mentioned.

Fig. 6. Mucin production by RbTE cells as a function of arotinoid concentration in the medium

Cells were cultured on collagen gels as described in the Experimental procedures section, with the indicated concentration of arotinoid present on days 3–12 after plating. [3 H]Glucosamine was added on day 12 for 48 h. The medium was subjected to chromatography on Sepharose Cl-4B and the resulting void-volume material was subjected to ion-exchange chromatography on DEAE-Sephacel. Mucin production is expressed as the amount of radioactivity unretarded by the ion-exchange column.

The effect of conditioned medium on mucin production suggests that mesenchymal factors, secreted by the 3T3 fibroblasts *in vitro* and possibly by underlying interstitial fibroblasts *in vivo*, play a role in enhancing tracheal mucin production. Again, the present data do not discriminate between the two competing hypotheses of stimulation of mucin production by cells already committed to mucosecretory function or the stimulation of differentiation toward such a phenotype. The latter interpretation has been applied to the effect of conditioned medium on the occurrence of ciliated cells in cultures of hamster tracheal epithelial cells (Lee *et al.*, 1984).

The increased mucin production observed upon inclusion of 8-bromo cyclic AMP in the RbTE cell medium can be interpreted either as a stimulation of secretion or as an augmentation of the differentiation process set in motion by retinoids. The involvement of cyclic AMP in mucin production has been observed in two other systems, one activated by prostaglandins (Marom *et al.*, 1981), the other by means of β -adrenergic receptors (Richardson *et al.*, 1978; Jones & Reid, 1982; Liedtke *et al.*, 1983). In each case, stimulus-secretion coupling is believed to occur in the submucosal glands

rather than the surface epithelium, either because the submucosa is the site of cyclic AMP accumulation (Lazarus *et al.*, 1984) or because it is the site of innervation (Das *et al.*, 1978).

Further, secretion stimulated by other agents (Jeffery, 1978; Sherman *et al.*, 1981) also results from submucosal secretory activity. Since the rabbit trachea contains very few submucosal glands (Breeze & Wheeldon, 1977), the RbTE cell-culture system can be assumed to be a model for secretion by surface epithelium. Although cyclic AMP accumulation mediated by both β -adrenergic receptors and prostaglandin E_1 has been documented in the rabbit trachea (Liedtke *et al.*, 1982) and localized to the epithelial layer, no stimulation of mucin secretion was observed (Liedtke *et al.*, 1983). Thus it seems unlikely that cyclic AMP enhancement of mucin production by RbTE cells *in vitro* is due to stimulation of secretion *per se*.

The alternative hypothesis, that cyclic AMP increases mucin production in a retinoid-dependent manner by influencing differentiation, is consistent with results in several other systems where retinoids and cyclic AMP act in concert to regulate the expression of differentiation-dependent functions. The systems where the effect has been observed are diverse: F9 embryonal carcinoma cells differentiating to yield parietal endoderm as measured by plasminogen-activator synthesis (Strickland & Mahdavi, 1978), U-937 monoblast-like cells differentiating to give monocyte-like cells as measured by the synthesis of an oxidase generating superoxide (Olsson & Breitman, 1982) and HL-60 leukaemic cells differentiating into granulocyte-like cells as measured by the induction of a specific transglutaminase (Davies *et al.*, 1985). Each system, however, shares the following attributes: (a) cyclic AMP analogues alone have no effect on the measured parameter; (b) retinoids alone induce at least a severalfold increase in the measured parameter; (c) retinoids plus cyclic AMP or its analogues increase the measured parameter 2–10-fold more than that seen with retinoids alone. Precisely the same effects on mucin production by RbTE cells are observed (Table 1). It is possible that retinoids induce either a cyclic AMP-dependent protein kinase or its substrate and that phosphorylation of this substrate enhances mucin production. Alternatively, retinoids may act to increase the interaction between control regions of genes and cyclic AMP-binding-protein complexes which have recently been proposed to regulate gene expression (Nagamine & Reich, 1985). The binding of both cyclic AMP-binding-protein complex and retinoid/retinoid-binding-protein complex (Chytil & Ong, 1979) to control regions may be required for optimal expression of some genes, including the ones mentioned above.

In summary, the secretion of mucins by RbTE cells in culture has been demonstrated and shown to be regulated by a number of factors. The system appears to facilitate the study of the regulation of mucin production by tracheal epithelium at the cellular level.

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