THE LOCALIZATION OF ACID MUCOPOLYSACCHARIDES IN THE GOLGI COMPLEX OF INTESTINAL GOBLET CELLS

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INTRODUCTION

Evidence for the role of the intestinal goblet cell in mucous secretion has been accumulating since 1837 (9). Nassonow (20) reported that mucous droplets of goblet cells first appear in the Golgi region of the cell, and this finding has been confirmed in recent years by numerous workers using a variety of experimental techniques (4–6, 10, 11, 15, 16, 21, 23, 24). However, the actual process by which mucus is formed by the goblet cell is still in doubt. The final secretory product is probably

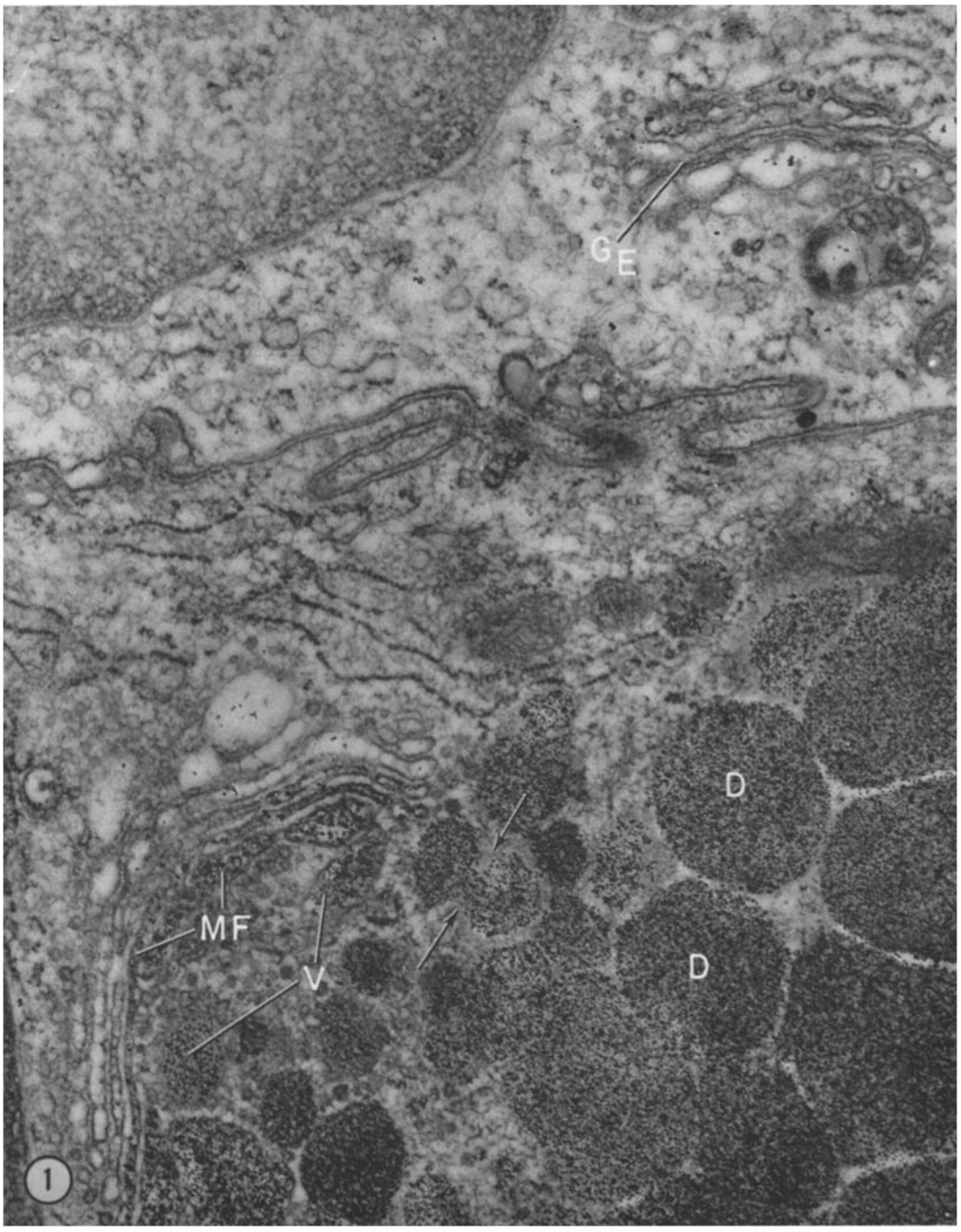

FIGURE 1 This micrograph of rat ileum shows portions of a goblet cell and an absorptive cell located near the villus tip. Thorium is localized in the cisternae at the mature face of the Golgi complex (*MF*), in Golgi vacuoles (*V*), and in mucous droplets (*D*) of the goblet cell. Possible coalescence of Golgi vacuoles are indicated (unlabeled arrows). Conversely, the Golgi complex of the epithelial cell (G_E) shows very few thorium particles. Other organelles in both cells show virtually no thorium staining. OsO_4-fixed methacrylate-embedded, and stained with thorium for acid mucopolysaccharides and with uranyl acetate and lead citrate to enhance contrast. × 35,000.

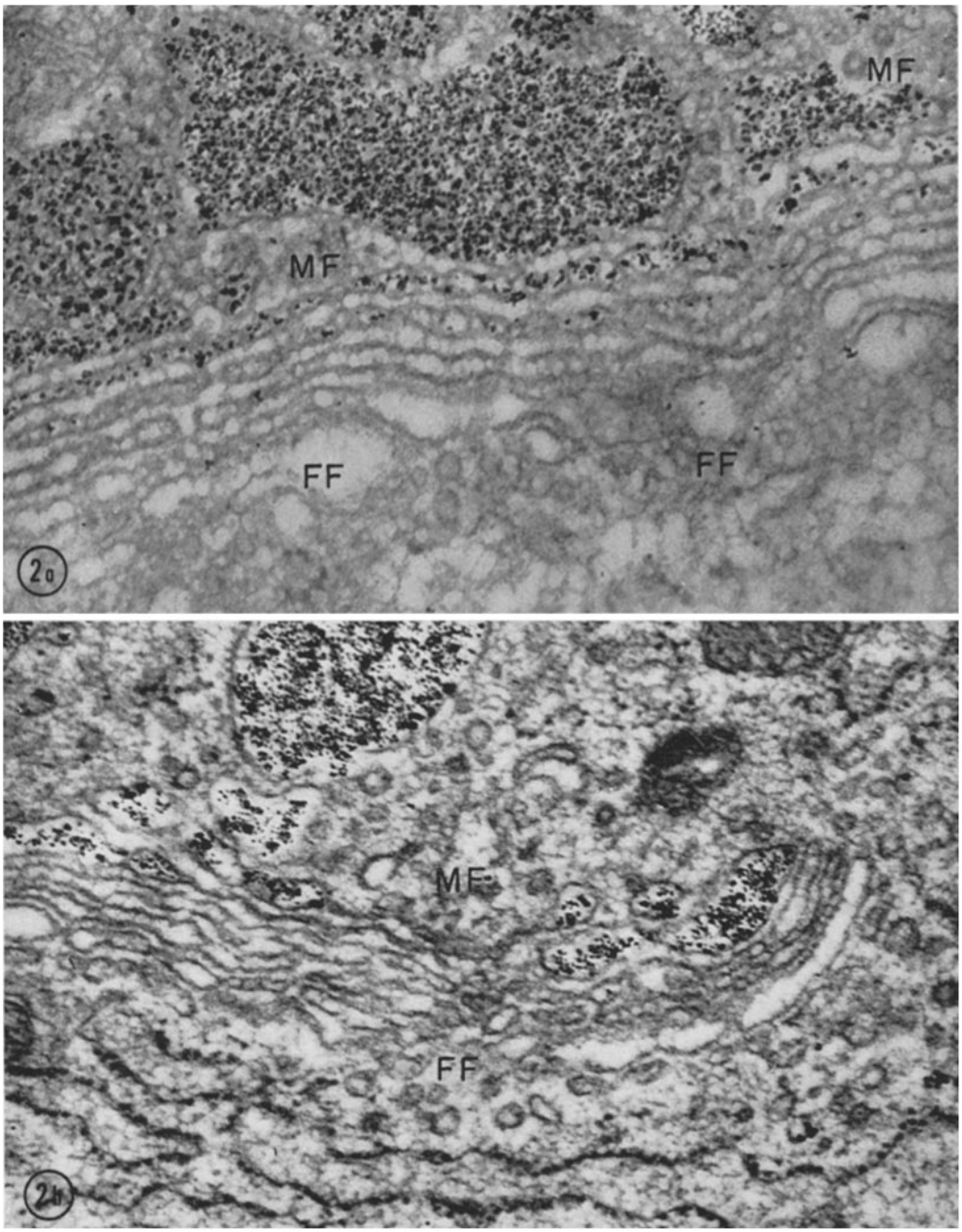

FIGURE 2 *a* Golgi complex of goblet cell from rat ileum. The thorium particles are localized in the intracisternal spaces at the mature face of the Golgi complex (*MF*), but not at the forming face (*FF*). Preparation similar to that of Fig. 1. × 76,000.

FIGURE 2 *b* Golgi complex of goblet cell from swine ileum. The localization of thorium is shown in the intracisternal spaces at the mature face (*MF*), but not at the forming face (*FF*). Preparation similar to that of Fig. 1. × 76,000.

complexed with a protein (21, 22), and Freeman (6) suggests that proteins synthesized by the endoplasmic reticulum are transported to the Golgi complex where they are combined with acid mucopolysaccharides and glycoproteins to form the mucous droplets visible in the electron microscope. The mucous droplets, as identified by their electron opacity, are first observed in the peripheral Golgi vacuoles and are not found in the parallel membranes comprising the Golgi complex proper (6). The present study utilized an acid mucopolysaccharide stain (26) at the electron microscope level to further determine the extent to which the Golgi complex is involved in mucous droplet formation in intestinal goblet cells.

MATERIALS AND METHODS

Tissue samples were excised from the terminal ileum of Charles River strain rats and Hanford-Labco miniature swine and preserved for electron microscopy in 0.067 M phosphate-buffered 1% osmium tetroxide at pH 7.3 for 1 hr at 4°C. After ethanol dehydration, the material was embedded in methacrylate (2). Individual villi, which were oriented longitudinally with respect to the future plane of sectioning, were trimmed so that the section face included only the apical ⅓–¼ of the villus. In addition, the extreme villus tip was trimmed off to eliminate cells which were in degenerative stages. Sections were obtained with diamond knives, expanded with xylene, and stained with colloidal thorium to demonstrate acid mucopolysaccharides (26). This was accomplished by floating the expanded sections on 30% acetic acid for 5 min, on 1% Thorotrast in 30% acetic acid for 5 min, on two changes of 30% acetic acid for 2 min each, and finally on distilled water. The sections were then placed on carbon-coated grids and subsequently double-stained with uranyl acetate and lead citrate to enhance contrast. The sections were examined with an RCA EMU-3B electron microscope.

RESULTS

The Golgi apparatus is a prominent structure in a mature goblet cell. It is composed of a stack of three or more parallel cisternae and usually exhibits a curved array (Fig. 1). The terms "forming face" and "mature face" have been applied to opposite sides of the Golgi complex by Mollenhauer and Whaley (19) and are suggestive of the polarized nature of this organelle as well as indicating its secretory function. The terms "forming face" and "mature face" will be used to describe the convex and concave sides, respectively, of the Golgi complex.

The Golgi cisternae at the forming face are dilated and their intracisternal spaces appear electron-transparent by the methods of preservation used in this study (Figs. 1, 2 *a* and *b*). Thorium particles, which are extremely electron opaque, are rarely observed within the cisternae at the forming face. The cisternae comprising the region between the forming face and the mature face are similar in appearance to the cisternae at the forming face except that they are rarely dilated (Figs. 1, 2 *a* and *b*). The cisternae at the mature face are not dilated, but are frequently observed to contain a substance of moderate electron opacity (Fig. 1) and are stained intensely with thorium (Figs. 1, 2 *a* and *b*). The thorium is found in the intracisternal spaces and does not appear to be attached to the limiting membrane. The cisterna at the extreme mature face is frequently dilated and appears to be forming Golgi vacuoles by a process of fragmentation (Figs. 1, 2 *b*). This cisterna and the Golgi vacuoles are also intensely stained with thorium (Figs. 1, 2 *a*). Suggestive evidence that the Golgi vacuoles coalesce with one another to form the larger mucous droplets that show uniform concentrations of thorium is shown in Fig. 1.

Despite the necessity to use methacrylate as the embedding medium for subsequent colloidal thorium staining (26), adequate preparations can be obtained in which cell fine structure is intact and reaction product sharly localized to specific cell components (Figs. 1, 2 *a* and *b*). The thorium particles, indicative of acid mucopolysaccharide, are localized in the cisternae at the mature face of the Golgi complex, in Golgi vacuoles adjacent to the mature face, and in mucous droplets of the goblet cells. The thorium was not associated with other cell organelles, i.e. rough endoplasmic reticulum, mitochondria, or nuclei. Also, concentrations of thorium were absent from adjacent epithelial cells although the Golgi regions of these cells did appear to contain slightly more thorium than could be explained by background (Fig. 1).

DISCUSSION

The preceding results were obtained from goblet cells located on the apical ⅓–¼ of rat and swine ileal villi, exclusive of those few cells which usually show cytoplasmic degeneration at the extreme tips of the villi. It is well known that the secretory potential of goblet cells increases as the cells migrate from the bottom of the crypts to the villus tip (17).

From this information, and from the morphological appearance of the goblet cells in the present investigation, it is concluded that the above descriptions are of mature, functioning goblet cells engaged in mucus synthesis and secretion.

The Golgi region of the intestinal goblet cell has been intimately connected to mucin synthesis and mucous droplet formation (4–6, 11). Radioautographic studies have clearly shown that the Golgi complex is capable of sulfation (15, 16) and have suggested that it is involved in glycoprotein, and possibly acid mucopolysaccharide, synthesis (21, 22, 24). The present investigation shows that the Golgi apparatus is involved in the synthesis of the acid mucopolysaccharide substance as well as in its intracellular transport (secretion). Furthermore, it would appear that the final synthesis of the acid mucopolysaccharide substance occurs primarily at the mature face of the Golgi complex. Whether this synthesis takes place in the intracisternal spaces or on the membranes limiting these spaces is unknown. While the thorium particles appear to be localized in the intracisternal spaces at the mature face of the Golgi complex, it is impossible, on the basis of these results, to rule out the cisternal membranes as playing a role in the synthesis of the secretory product and the subsequent transfer of the product to the intracisternal spaces.

The staining mechanism of acid mucopolysaccharides by colloidal thorium is unknown. Revel (26) suggests that the staining action results from the interaction of positively charged colloidal micelles with strong acidic groups, such as uronic carboxyl and/or sulfate carried by acid mucopolysaccharides. Although it is presently impossible to equate the sulfation which is known to occur in the Golgi apparatus of these cells (15, 16) with thorium staining, it seems likely that these two phenomena may be related. If so, then sulfation of acid mucopolysaccharides is localized in the cisternae at the mature face of the Golgi complex.

Many studies have shown the existence of a morphological and a functional polarity of the Golgi complex in a variety of plant and animal cells (1, 3, 7, 8, 19, 25, 27). The present investigation substantiates and extends our knowledge of the Golgi apparatus. It is now possible to suggest that the Golgi complex, of the intestinal goblet cell, at least in the villous region examined in this study, is composed of three, and possibly four, distinct morphological regions and that each region has a separate function. These regions are: (1) The forming face characterized by dilated cisternae containing no visible electron-opaque materials. There is good reason to believe that these cisternae function as a collection point for various materials, some of which are probably synthesized by the rough endoplasmic reticulum (6). (2) The midregion zone, located between the forming face and the mature face, where the cisternae are not dilated and intracisternal material, as recognized by its electron opacity, is not present. The functional role of this region is not clear, but it may involve condensation of the various materials collected at the forming face. (3) The mature face with nondilated cisternae which frequently contain a substance of moderate electron opacity. The localization of thorium within these cisternae is considered as evidence that the materials may be sulfated at this location; certainly the materials are chemically modified in such a way as to give a reaction product with low pH thorium staining. (4) There may be a region, immediately adjacent to the mature face, composed of fragmenting cisternae which are in the process of forming Golgi vacuoles containing the secretory product. In summary, the secretory role of the Golgi complex in the intestinal goblet cell on the apical $\frac{1}{3}$–$\frac{1}{4}$ of rat and swine ileal villi, and perhaps in other cells as well, appears to involve collection, condensation, modification, and preparation of the secretory product for further transport. In addition, the occurrence of these processes at different locations within the Golgi apparatus implies a functional specialization within the elements of the Golgi complex.

Whether the goblet cell and the absorptive cell represent different cell lines or whether they are derived from a common stem cell remains an open and tantalizing question. It would be interesting to know whether or not the supposed progenitor cells of both goblet cells and absorptive cells, i.e. the undifferentiated cells in the depths of the crypts, have Golgi complexes containing acid mucopolysaccharides. Unfortunately, the crypt cells were not examined in this investigation. That the Golgi complexes of absorptive cells were slightly stained with colloidal thorium might suggest that these cells retain the potential of a "stem cell" to become a secretory (goblet) cell or that these cells are goblet cells in a postsecretory phase. The former possibility would offer a satisfactory explanation for the increased proportion of goblet cells that occurs on villi in the absence of bile or

during inflammation (18). However, Ito and Revel (14) have shown that precursors of the fuzzy coat occur in the Golgi region of the absorptive cell. The fuzzy coat is known to have an acid mucopolysaccharide component (12, 13, 18), and the occurrence of thorium particles in the Golgi complex of absorptive cells would appear to represent fuzzy coat precursor materials rather than "stem cell" potential.

SUMMARY

A colloidal thorium stain was utilized at the electron microscope level to demonstrate acid mucopolysaccharides in intestinal goblet cells. The acid mucopolysaccharide substance was localized in the intracisternal spaces at the mature face of the Golgi complex, in Golgi vacuoles, and the mucous droplets. The cisternae at the forming face of the Golgi apparatus did not contain acid mucopolysaccharides, as evidenced by their inability to bind thorium. In addition to a secretory function, this finding suggests that the Golgi complex is also involved in synthesizing the final secretory product of the goblet cell. This synthesis, which possibly includes sulfation of the acid mucopolysaccharide portion of the mucus, occurs primarily, if not exclusively, in the cisternae at the mature face of the Golgi complex. Such localization is further evidence for the existence of a functional as well as a morphological polarity in the Golgi apparatus. It is suggested that the Golgi complex of the goblet cell is composed of three distinct morphological and functional regions: the forming face, a mid-region, and the mature face.

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