CYTOCHEMICAL STAINING OF THE GOLGI APPARATUS IN *AMOEBA PROTEUS*

GARY E. WISE and CHARLES J. FLICKINGER. From the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80302

The Golgi apparatus has been shown to be involved in the packaging of secretory proteins (Caro and Palade, 1964; Jamieson and Palade 1967 *a*, 1967 *b*), and in the production of acid mucopolysaccharides and glycoproteins (Neutra and Leblond, 1966; Berlin, 1967; Thiéry, 1967; Rambourg et al, 1969; Whur et al., 1969). Other suggested functions of the Golgi apparatus include the formation of the plasma membrane

(Hicks, 1966; Rambourg et al., 1969) and primary lysosomes (Novikoff et al., 1963; Novikoff et al., 1964; Bainton and Farquhar, 1966; Friend and Farquhar, 1967), as well as the packaging of other cell products (see reviews by Mollenhauer and Morré, 1966; Beams and Kessel, 1968).

In most studies, one function of the Golgi apparatus has usually been emphasized for a

given cell type, but it is possible that the Golgi membranes of individual cells are capable of multiple functions. Although Golgi complexes in the differentiated cells of multicellular organisms may often be specialized to perform largely or exclusively one function, unicellular organisms may provide more frequent examples of multiple functions performed by the Golgi membranes coexisting within the same cell. The role of the Golgi apparatus in amebae is of interest in this regard, because amebae are capable of several activities that have been related to the functioning of the Golgi complex. For example, amebae are able to renew their cell surface within a few hours (e.g., Wolpert and O'Neill, 1962; Nachmias, 1966), and the Golgi apparatus has been implicated in the production of new plasma membrane and cell coat (Stockem, 1969, Wise and Flickinger, 1970). In addition, material ingested by pinocytosis or phagocytosis probably is digested by acid hydrolases in large lysosomes or food vacuoles (e.g., Chapman-Andresen and Lagunoff, 1966), and the primary lysosomes might originate from the Golgi apparatus.

In the present investigation, several histochemical reactions were used to study the Golgi complexes of *Amoeba proteus*. Within individual stacks, the cisternae at the convex pole of the stack possessed acid phosphatase and thiamine pyrophosphatase activity. Those cisternae at the concave pole lacked the enzymatic activity but stained for the presence of glycoproteins.

MATERIALS AND METHODS

Preparative Procedure for Electron Microscopy

Amebae were fixed at room temperature for 30–45 min in Karnovsky's mixture of 5% glutaraldehyde and 4% paraformaldehyde (Karnovsky, 1965). The cells were rinsed three times and then washed overnight in distilled water so as to rid the cells of cytoplasmic crystals. Following incubation in the histochemical medium, cells were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer at pH 7.3 for 30 min. Next, they were dehydrated in a graded series of ethanols, immersed in propylene oxide and embedded in Araldite. Silver-to-pale-gold sections were cut on a Porter-Blum MT-2 ultramicrotome and were either stained with lead citrate or were viewed unstained with a Phillips 300 electron microscope.

Acid Phosphatase (AcPase) Method

Aldehyde-fixed amebae were placed for 15 min in an incubation medium (prewarmed to 37°C) composed of 1 volume 1.25% Na β-glycerophosphate (Nutritional Biochemicals Corp., Cleveland, Ohio) solution of pH 5.0, 1 volume of distilled water, 1 volume of 0.2 M Tris-maleate buffer (pH 5.0), and 2 volumes of 0.2% lead nitrate (Barka and Anderson, 1962). After incubation, the amebae were rinsed in three 5-min washes of distilled water, 1% acetic acid, and distilled water before being postfixed. Controls consisted of leaving out the substrate or adding 0.01 M NaF to the incubation medium.

Thiamine Pyrophosphatase (TPPase) Method

After fixation in aldehydes, amebae were immersed for 10 or 15 min at 37°C in an incubation medium consisting of 5 volumes of 0.01 M thiamine pyrophosphate (Sigma Chemical Company, St. Louis, Mo.), 2 volumes of distilled water, 10 volumes of 0.2 M Tris-maleate buffer (pH = 7.2), 5 volumes of 0.025 M $MnCl_2$, and 3 volumes of 0.2% lead nitrate (modified after Novikoff and Goldfischer, 1961). After incubation, the amebae were rinsed in three 5-min washes as described above. Controls consisted of omitting thiamine pyrophosphate and $MnCl_2$ from the medium.

Periodic Acid–Silver Methenamine Technique

Complex carbohydrates were localized by a technique devised by Rambourg (1967). Details of the periodic acid oxidation followed by silver methenamine staining were outlined in a previous paper (Wise and Flickinger, 1970).

RESULTS

Untreated Amebae

The multiple Golgi complexes of normal amebae are composed of a stack of six to eight curved parallel cisternae about 1–1.5 μ in diameter or lateral extent (Fig. 1). The ends of the cisternae often are more expanded at the concave pole of the stack than at the convex pole. One or two cisternae at the concave edge of the array are sometimes dilated throughout their extent and frequently contain a moderately dense, filamentous material. Morphologically similar filaments coat the outer surface of the plasma membrane and line the inside of a multitude of cytoplasmic vacuoles. Small numbers of smooth vesicles 400 A in diameter are distributed around the periphery of the Golgi cisternae. Golgi complexes are scattered throughout the cytoplasm,
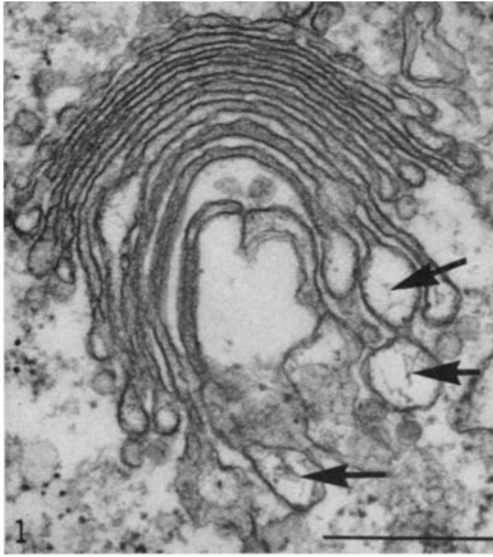
The length of the bar in each micrograph is 0.5 μ.
FIGURE 1 Normal, unstained Golgi apparatus illustrating the polarity in its organization and the filamentous material (arrows) in the dilated cisternae of the concave face. × 45,000.

and it is estimated that the average ameba contains several hundred of these organelles.

Acid Phosphatase

Acid phosphatase reaction product was observed in two to five cisternae at the convex side of virtually all Golgi complexes (Fig. 2). In contrast, the remaining three to five cisternae toward the concave pole, which often contained filamentous material, displayed no electron-opaque reaction product (Fig. 2). Vesicles distributed around the convex pole of the Golgi apparatus also contained acid phosphatase reaction product.

Occasionally, some reaction product was observed in the lumen of the granular endoplasmic reticulum. The intensity of the reaction, when present, was usually less than in the Golgi cisternae. Reaction product was never observed in the agranular endoplasmic reticulum.

The addition of 0.01 M NaF to the incubation medium resulted in almost complete suppression of the reaction. Controls with NaF uniformly contained very little or no electron-opaque reaction product. Controls in which the substrate was omitted were not always as consistent in

appearance and exhibited generally reduced but varying amounts of electron-opaque material.

Thiamine Pyrophosphatase

The distribution of thiamine pyrophosphatase reaction product closely paralleled that of acid phosphatase. Only three to five cisternae at the convex pole of the Golgi complexes contained reaction product, while the remainder of the cisternae toward the concave pole were devoid of activity (Fig. 3). A small amount of reaction product was occasionally present in parts of the granular endoplasmic reticulum. No TPPase activity was detected in the agranular reticulum.

Controls in which substrate and $MnCl_2$ were omitted from the medium were devoid of reaction product (Fig. 4).

Localization of Complex Carbohydrates with Periodic Acid–Silver Methenamine Stain

The electron-opaque deposits of silver were localized in the cisternae at the concave side of the Golgi complex (Fig. 5), in association with the filamentous surface coat, and in cytoplasmic vacuoles lined with filamentous material, as previously reported (Wise and Flickinger, 1970). The distribution of electron-opaque material corresponded to the presence of filamentous material in the Golgi complex. Thus, the expanded ends of cisternae and the dilated cisternae at the concave pole were stained, in contrast with the localization of phosphatase reaction product in cisternae at the convex pole.

In control sections some silver was present, imparting a finely granular appearance to much of the section (Fig. 6). These deposits were not localized or concentrated over any parts of the cells, but did predominate in randomly distributed areas where the Araldite appeared to have been leached out or thinned during the staining process. A similar background was visualized in the experimental sections.

DISCUSSION

The results of these histochemical studies indicate that, within each stack, ameba Golgi cisternae exhibit regional differences in their chemical composition. The cisternae on the convex side of the Golgi apparatus contain acid phosphatase and thiamine pyrophosphatase, whereas the cisternae on the concave side contain glyco-
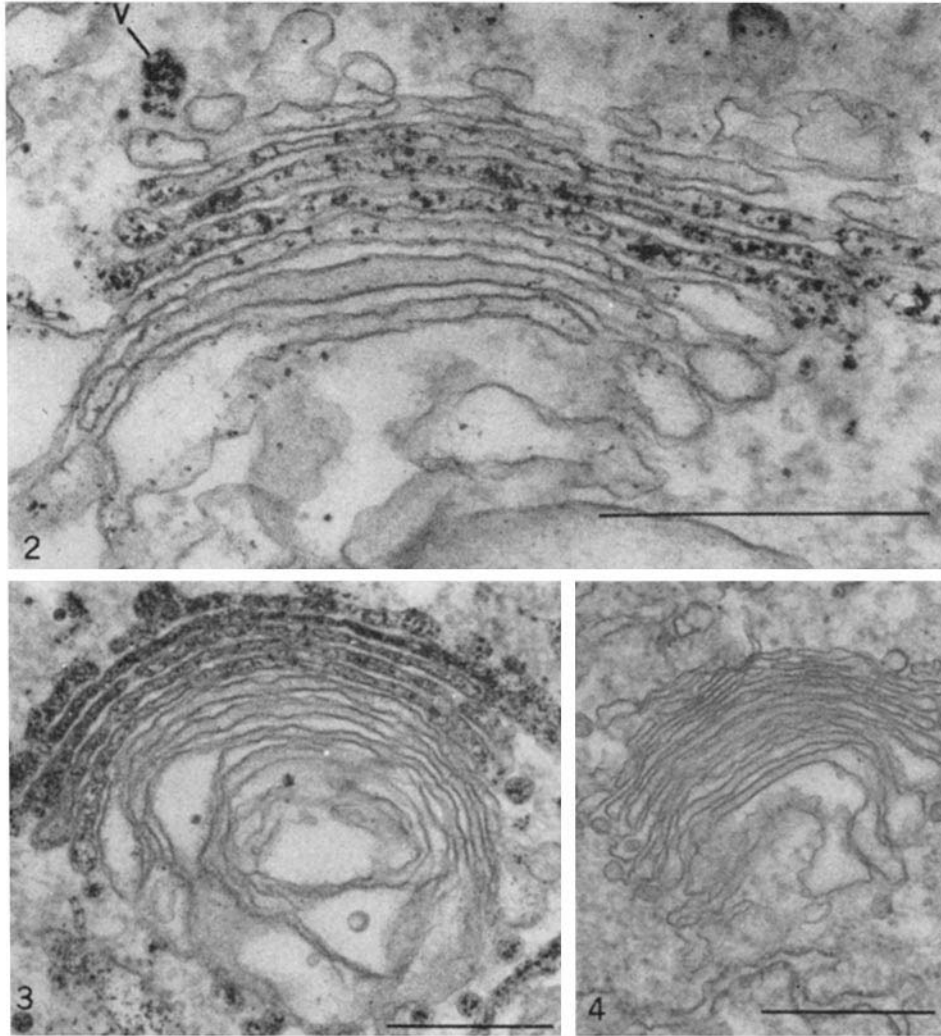
FIGURE 2   Ameba incubated in Na β-glycerophosphate for the detection of AcPase. Reaction product is present in the cisternae of the convex face of the Golgi apparatus and in vesicles (V) which are occasionally seen near the convex pole. × 87,500.

FIGURE 3   Ameba incubated in thiamine pyrophosphate for the presence of TPPase. Reaction product is present in five cisternae on the convex face of the Golgi apparatus, similar to the distribution of AcPase. × 54,000.

FIGURE 4   Control for TPPase in which substrate is omitted. Reaction product is absent from the Golgi apparatus and granular endoplasmic reticulum. × 45,000.

proteins. It is uncertain whether any cisternae in the center of Golgi complexes contain both phosphatase and glycoproteins. These chemical differences between Golgi cisternae may, in turn, reflect functional differences between Golgi cisternae in different locations within the organelle. Although the functions of ameba Golgi complexes have not definitely been proved, available evidence suggests that they may participate in the manufacture of the cell surface coat (Stockem, 1969; Wise and Flickinger, 1970). This activity may be carried out by the cisternae at the concave side that stain for glycoproteins. In contrast, the presence of acid phosphatase and thiamine
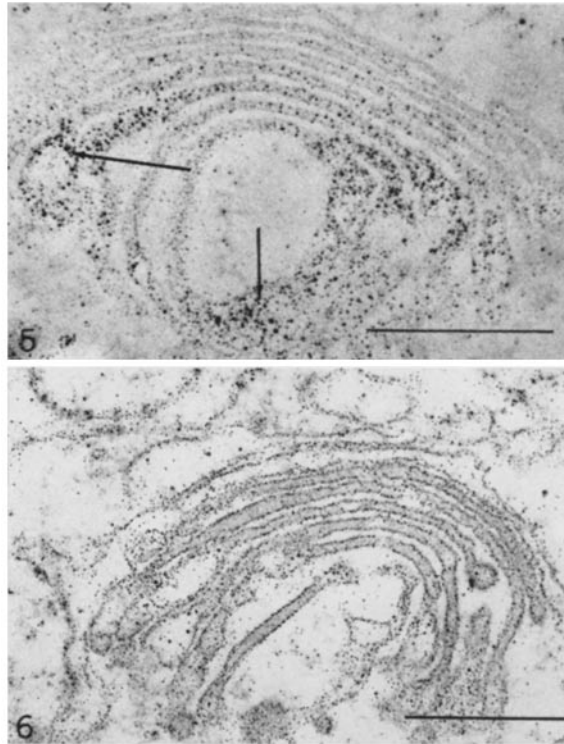
FIGURE 5 Periodic acid–silver methenamine stain is concentrated in the cisternae of the concave face (arrows) of the Golgi apparatus. × 49,500.

FIGURE 6 Silver methenamine control illustrating reduction of staining in the cisternae of the concave pole of the Golgi apparatus. × 42,000.

pyrophosphatase activity in the cisternae at the convex pole implies that these cisternae may have a different action. In other cells, the presence of acid phosphatase activity in Golgi cisternae has been linked to the packaging of hydrolases to form primary lysosomes (see review by Novikoff, 1967 b). Similarly, this may be the role of the cisternae at the convex side of Golgi complexes in amebae.

Polarity in the histochemical reactions of Golgi complexes in other cells has previously been reported. The presence of a histochemical reaction in some Golgi cisternae and its absence in other cisternae have usually been described, rather than the presence of two different reactions, as in the present study. Phosphatases have been detected in cisternae at one side of the Golgi complex in some cases (Smith and Farquhar, 1966; Novikoff, 1967 a; Bertolini and Hassan, 1967; Friend and Farquhar, 1967; Dauwalder et al., 1969). Similarly, in different instances, the presence of polysaccharide-rich materials

in some but not all Golgi cisternae has been described (e.g., Rambourg et al. 1969).

According to one hypothesis (Mollenhauer and Whaley, 1963), the Golgi complex is a dynamic organelle, and a cisterna moves through the Golgi apparatus from forming face to mature face while it undergoes gradual progressive modification in form and presumably also in content and chemical composition. The concept of forming and mature faces, however, may require modification or qualification in some instances. For example, Bainton and Farquhar (1966) have shown that in developing polymorphonuclear leukocytes two different types of granules (azurophil and specific) are produced from opposite sides of the Golgi complex at sequential times during development. It appears either that the direction of movement of cisternae through the Golgi complex reverses during development, or that these Golgi complexes are bipolar and capable of releasing a product from either pole.

In the present study, phosphatases were lo-

calized at one pole and glycoproteins at the other, and these substances are present simultaneously, unlike the two types of granules in PMN leukocytes. If cisternae actually move through ameba Golgi complexes, then a cisterna must not only lose or inactivate phosphatases but also activate enzymes for synthesizing or concentrating glycoproteins (the reverse sequence is equally possible). This implies that an individual cisterna is capable of different activities at different times during its progress through the Golgi complex. Alternatively, perhaps ameba Golgi complexes are two functional units that are closely associated morphologically. If this is the case, there might be little exchange of membranes between the two parts, and each part might turn over individually at the same or at different rates. In addition, the transport of substances into the Golgi complex might occur at different points along its surface rather than being restricted to one pole. Although the granular endoplasmic reticulum forms its most intimate association with the Golgi complex at the convex pole, transitional elements containing both rough and smooth regions were observed in proximity to the ends of Golgi cisternae throughout the stack.

No heterogeneity among Golgi complexes was detected with the methods used in this study. Instead, all the Golgi complexes within an ameba reacted in a similar fashion and stained with approximately the same intensity. This does not exclude the possibility, of course, that other enzymes or compounds might be distributed unequally amongst Golgi complexes.

REFERENCES

1. BAINTON, D. F., and M. G. FARQUHAR. 1966. Origin of granules in polymorphonuclear leukocytes. Two types derived from opposite faces in the Golgi complex in developing granulocytes. *J. Cell Biol.* **28**:277.
2. BARKA, T., and P. J. ANDERSON. 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* **10**:741.
3. BEAMS, H. W., and R. G. KESSEL. 1968. The Golgi apparatus: Structure and function. *Int. Rev. Cytol.* **23**:209.
4. BERLIN, J. D. 1967. The localization of acid mucopolysaccharides in the Golgi complex of intestinal goblet cells. *J. Cell Biol.* **32**:760.
5. BERTOLINI, B., and G. HASSAN. 1967. Acid phosphatase associated with the Golgi apparatus in human liver cells. *J. Cell Biol.* **32**:216.
6. CARO, L. G., and G. E. PALADE. 1964. Protein synthesis, storage, and discharge in the pancreatic exocrine cell. An autoradiographic study. *J. Cell Biol.* **20**:473.
7. CHAPMAN-ANDRESEN, C., and D. LAGUNOFF. 1966. The distribution of acid phosphatase in the amoeba *Chaos chaos* L. *C. R. Trav. Lab. Carlsberg.* **35**:419.
8. DAUWALDER, M., W. G. WHALEY, and J. E. KEPHART. 1969. Phosphatases and differentiation of the Golgi apparatus. *J. Cell Sci.* **4**:455.
9. FRIEND, D. S., and M. G. FARQUHAR. 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* **35**:357.
10. HICKS, R. M. 1966. The function of the Golgi complex in transitional epithelium: Synthesis of the thick cell membrane. *J. Cell Biol.* **30**:623.
11. JAMIESON, J. D., and G. E. PALADE. 1967 *a.* Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. *J. Cell Biol.* **34**:577.
12. JAMIESON, J. D., and G. E. PALADE. 1967 *b.* Intracellular transport of secretory proteins in the pancreatic exocrine cell. II. Transport of condensing vacuoles and zymogen granules. *J. Cell Biol.* **34**:597.
13. KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**: 137A. (Abstr.)
14. MOLLENHAUER, H. H., and D. J. MORRÉ. 1966. Golgi apparatus and plant secretion. *Ann. Rev. Plant Physiol.* **17**:27.
15. MOLLENHAUER, H. H., and W. G. WHALEY. 1963. An observation on the functioning of the Golgi apparatus. *J. Cell Biol.* **17**:222.
16. NACHMIAS, V. T. 1966. A study by electron microscopy of the formation of new surface by *Chaos chaos*. *Exp. Cell Res.* **43**:583.
17. NEUTRA, M., and C. P. LEBLOND. 1966. Synthesis of the carbohydrate of mucus in the Golgi com-

plex as shown by electron microscope radio-autography of goblet cells from rats injected with glucose-H[3]. *J. Cell Biol.* **30:**119.

18. Novikoff, A. B., and S. Goldfischer. 1961. Nucleosidediphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. *Proc. Nat. Acad. Sci. U.S.A.* **47:**802.

19. Novikoff, A. B., E. Essner, and N. Quintana. 1963. Relations of endoplasmic reticulum, Golgi apparatus and lysosomes. *J. Microsc.* **2:**3.

20. Novikoff, A. B., E. Essner, and N. Quintana. 1964. Golgi apparatus and lysosomes. *Fed. Proc.* **23:**1010.

21. Novikoff, A. B. 1967 *a*. Enzyme localization and ultrastructure of neurons. *In* The Neuron. H. Hyden, editor. Elsevier Publishing Co., New York. 255.

22. Novikoff, A. B. 1967 *b*. Lysosomes in nerve cells. *In* The Neuron. H. Hyden, editor. Elsevier Publishing Co., New York. 319.

23. Rambourg, A. 1967. An improved silver methenamine technique for the detection of periodic acid-reactive complex carbohydrates with the electron microscope. *J. Histochem. Cytochem.* **15:**409.

24. Rambourg, A., W. Hernandez, and C. P.

Leblond. 1969. Detection of complex carbohydrates in the Golgi apparatus of rat cells. *J. Cell Biol.* **40:**395.

25. Smith, R. E., and M. G. Farquhar. 1966. Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland. *J. Cell Biol.* **31:**319.

26. Stockem, W. 1969. Pinocytose und bewegung von amöeben. III. Die funktion des Golgi apparates von *Amoeba proteus* und *Chaos chaos*. *Histochemie.* **18:**217.

27. Thiéry, J. P. 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microsc.* **6:**987.

28. Whur, P., A. Herscovics, and C. P. Leblond. 1969. Radioautographic visualization of the incorporation of galactose-^{3}H and mannose-^{3}H by rat thyroids in vitro in relation to the stages of thyroglobulin synthesis. *J. Cell Biol.* **43:**289.

29. Wise, G. E., and C. J. Flickinger. 1970. Relation of the Golgi apparatus to the cell coat in amoebae. *Exp. Cell Res.* In press.

30. Wolpert, L., and C. H. O'Neill. 1962. Dynamics of the membrane of *Amoeba proteus* studied with labeled specific antibody. *Nature (London).* **196:**1261.