A GOLGI APPARATUS ASSOCIATED WITH MATING IN *TETRAHYMENA PYRIFORMIS*

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ABSTRACT

The classical Golgi apparatus has not been observed in the several strains of *Tetrahymena pyriformis* examined in this laboratory at the ultrastructural level when the ciliates are grown vegetatively. However, sexually active strains, when starved for the purpose of inducing conjugation, contain stacked saccules in the oral region. When such opposite mating types are mixed for mating, the stacked saccules become swollen at their ends and vesicles appear to pinch off from them. These bodies possess the configuration of the classical Golgi apparatus of other eucells. Vesicles seem to be formed from the saccules just prior to, and toward the end of conjugation, suggesting a relationship with the mating process.

INTRODUCTION

Most protozoa contain a well defined Golgi apparatus consisting of stacked, smooth, flattened saccules with swollen ends and small vesicles close by (11, 20). The ciliates, however, do not present such a typical picture of the Golgi apparatus. In spite of numerous ultrastructural studies on this group of protozoa, the Golgi apparatus has been described in only a few. Until recently, none has been observed in the several thousand micrographs taken of *Tetrahymena pyriformis* in our laboratory. However, during certain stages in the life cycle a well defined Golgi apparatus has been observed. The purpose of this communication is to describe this structure and the conditions under which it is induced.

MATERIALS AND METHODS

Sexually active strains of *T. pyriformis*, variety 1, mating types I and II[1] were used in this investigation. They were grown axenically for 3 days (log growth phase) in 500-ml Erlenmeyer flasks containing 100 ml of modified peptone medium (6). When starvation was under investigation, the ciliates were axenically washed and resuspended in a sterile inorganic buffer solution (4). For studies involving starvation alone, strain B16652e (mating type II) was used exclusively. For conjugation studies, both mating types were starved for 12–48 hr in buffered salt solution and then mixed. At specified intervals during conjugation, samples were withdrawn and prepared for electron microscopy.

The cells in all experiments were concentrated by centrifugation into soft pellets and fixed in either cold 1% osmium tetroxide buffered at pH 7.4 with a phosphate salt solution (21) for 20 min, or in cold 2% glutaraldehyde buffered to pH 7.2 with the phosphate salt solution, or with 0.05 M sodium cacodylate for 15 min according to the method of Gordon et al. (10). The ciliates fixed in glutaraldehyde in phosphate buffer were washed in cold phosphate solution and postfixed for 15 min in cold 1% osmium tetroxide buffered to pH 7.4 with the same phosphate solution. Cells fixed in glutaraldehyde with cacodylate buffer were postfixed for 1 hr at room temperature in 1% osmium tetroxide in 0.14 M veronal-acetate buffer (pH 7.4) containing 0.13 M sucrose.

[1] Strains A15651c and B16652e, mating types I and II, respectively, were provided through the courtesy of Dr. Sally Allen, Department of Zoology, University of Michigan, Ann Arbor, Mich.

Acid phosphatase localization was accomplished by employing the Gomori technique (9) with modifications. The ciliates were briefly prefixed in cold 2% glutaraldehyde buffered with 0.05 M sodium cacodylate, followed by three changes of cold 0.08 M sodium cacodylate containing 0.18 M sucrose (pH 5.8). They were then incubated for 1½ hr at room temperature in an incubation medium consisting of 0.1 M sodium β-glycerophosphate and 0.003 M lead acetate buffered to pH 5.8 with 0.05 M acetate buffer. This solution was mixed fresh, heated to 60°C for 1 hr, cooled, and filtered before use (16). The incubation suspension was shaken throughout the incubation period, after which the cells were rinsed twice, for less than 30 sec in each change, in a 1% solution of acetic acid containing 0.13 M sucrose. These brief rinses were interrupted with quick changes of acetate buffer. Controls were handled in the same way except that the substrate was omitted from the incubation medium. The cells were then postfixed for 1 hr in 1% osmium tetroxide buffered with 0.14 M veronal-acetate (pH 7.4) containing 0.13 M sucrose.

Dehydration was carried out in a graded series of ethanol. Cells were finally dehydrated and cleared in either acetone or propylene oxide before being embedded in Epon 812 according to the method of Luft (14). Polymerization was achieved at 60°C. The specimens were sectioned on a Porter-Blum MT2 microtome with either glass or diamond knives and stained with either uranyl acetate alone or uranyl acetate followed by lead citrate (22). Thin sections were collected on naked or Formvar-coated carbon-stabilized grids and were examined with an RCA EMU 3E electron microscope operated at 50 kv and provided with a 25-μ objective aperture.

OBSERVATIONS

Ciliates in logarithmic growth contain numerous, separate, smooth, flattened saccules in the oral region (Fig. 1). The saccule membranes are dense and the distance between them is uniform (25 mμ). The saccules exist in an array of complete ovals, V's, and straight or wavy patterns. They are never stacked, and no terminal swellings or vesicles are observed. Such saccules are rarely seen elsewhere in the ciliate. Faintly dense tubules with a diameter of approximately 200 mμ lie among the saccules. Their identity is obscure.

When these cells are tested for the presence of acid phosphatase, no reaction product appears in the saccules (Fig. 2), suggesting that hydrolases are not produced or stored in them. Nearby lysosomes show reaction products, indicating that these bodies contain digestive enzymes.

When ciliates are starved for 12–48 hr in buffered salt solution prior to mixing the cells for mating, the saccules become aligned in stacks consisting of a few to several lamellae (Fig. 3). Few, if any, vesicles are seen in the vicinity of these structures, suggesting that no products are being packaged by them. When these starved ciliates are mixed with an opposite mating type, conjugation occurs within 2–3 hr. Cells that have been mixed for as short a time as 5 min show more complex structures in the mouth region of the ciliate (Fig. 4). The ends of the stacked saccules are enlarged throughout most of their length, and numerous vesicles lie in the immediate vicinity. A vacuole containing whorls of dense membranes is usually associated with this structure. It may be a residual vacuole.

Once the conjugants become firmly fused (7 hr after mixing), some of the saccules near the region of contact between the mates are arranged in stacks of several lamellae (Fig. 5). Other saccules are randomly distributed as single strands or as complete ovals and circles. They seem to be more numerous in one of the mates than the other but we have been unable to determine whether or not this is consistently true. No vesicles appear near the saccules, suggesting that nothing is being packaged. Later in conjugation (10 hr after mixing) some of the stacked saccules possess terminal swellings, and numerous vesicles lie near by (Fig. 6). Other stacked saccules are without swollen ends or vesicles. When ciliates in this stage are tested for the presence of acid phosphatase, no reaction product appears in the saccules, or in the vesicles indicating that the substance being packaged is not a hydrolase.

Toward the end of conjugation (20 hr after mixing) or just after the conjugants separate, the stacked saccules, and associated vesicles, are retained (Fig. 7). They appear as concentric whorls in some profiles (Fig. 8). When these cells undergo fission, following a period of feeding, the saccules resume the configuration observed in cells in logarithmic growth (Fig. 1).

DISCUSSION

The classical Golgi apparatus has been described in representatives of all the major groups of protozoa (11, 20, 25). Whereas it is conspicuous in some groups, the flagellates for example (12, 20), it has been reported in only a few ciliates. It was first described in several representatives of the family Ophryoscolecidae (17) and more recently in *Epistylis anastatica* (7), *Blepharisma undulans* (13), and *Paramecium multimicronucleatum* (21).

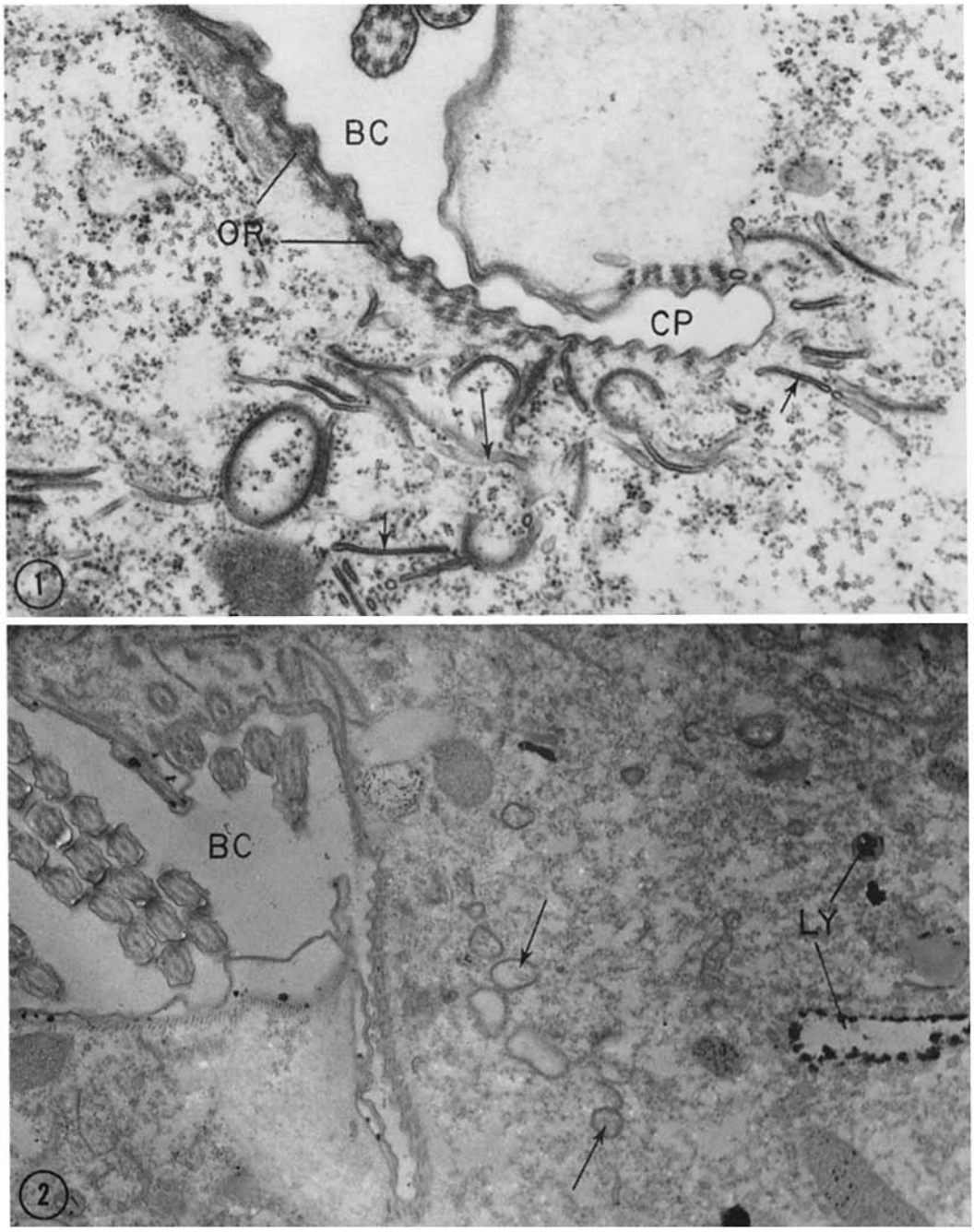
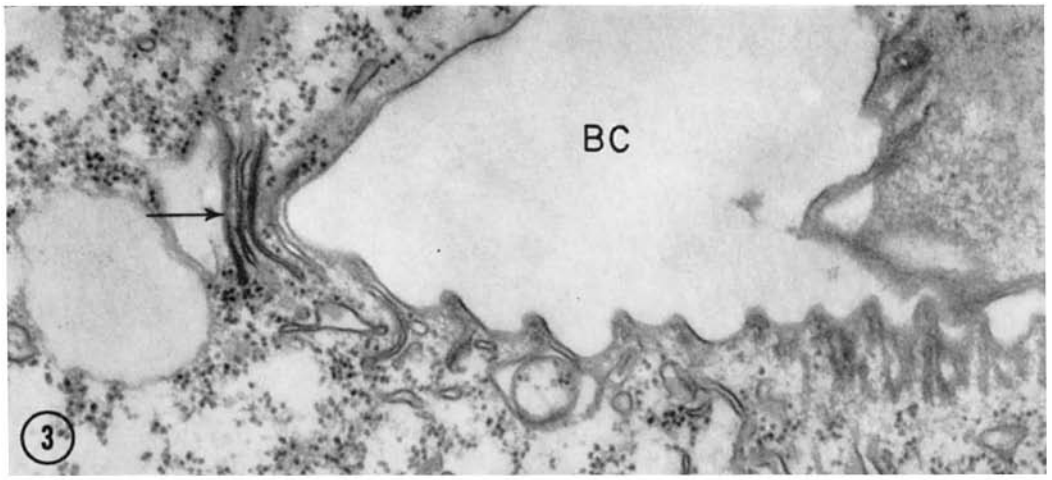


FIGURE 1 A section through the buccal cavity of a ciliate that was in logarithmic growth, showing dense-membraned, flattened saccules (short arrows) and less dense tubules (long arrow). The buccal cavity (*BC*), oral ribs (*OR*), and cytopharynx (*CP*) also show in the micrograph. Fixed in glutaraldehyde; postfixed in OsO_4. × 37,500.

FIGURE 2 A section, similar to Fig. 1, but treated for the presence of acid phosphatase. No reaction product appears in the saccules (arrows) but does occur in the lysosome (*LY*). Fixed in glutaraldehyde; postfixed in OsO_4. × 20,000.



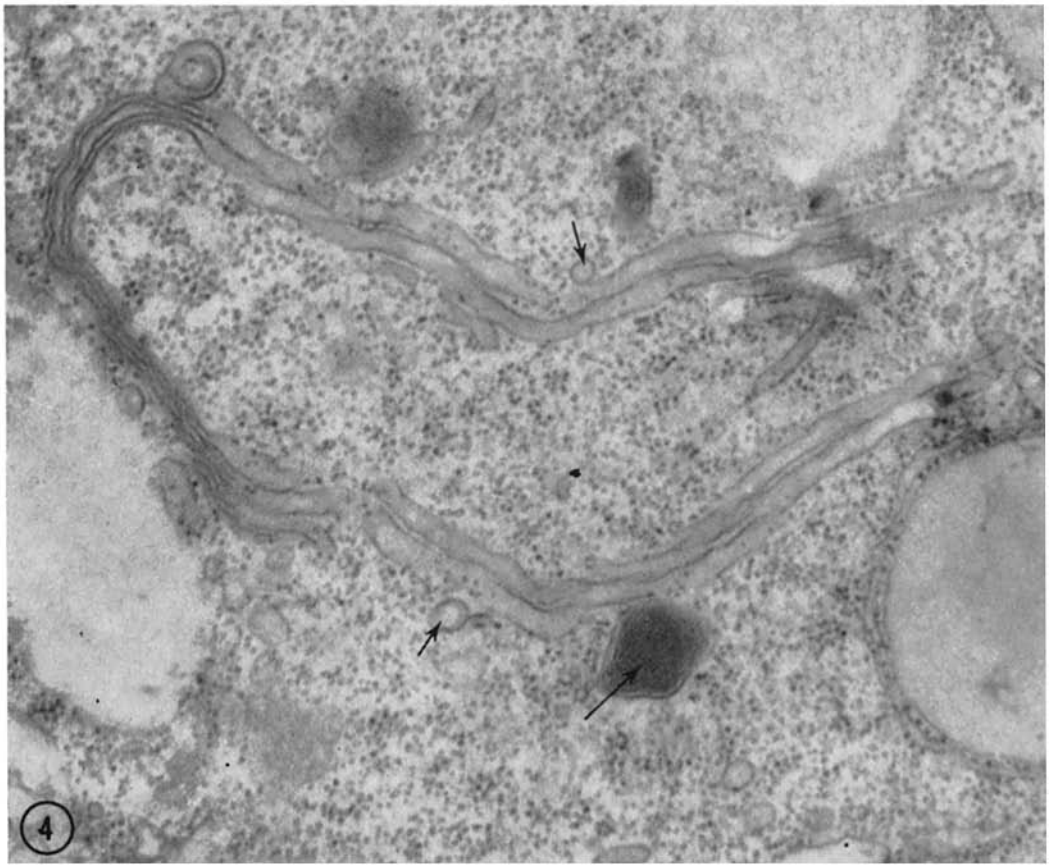


FIGURE 3 A section through the mouth of a ciliate that has been starved for 48 hr, showing three stacked saccules (arrow). Other saccules are oriented randomly as in logarithmic growing cells. Note buccal cavity (*BC*). Fixed in glutaraldehyde; postfixed in OsO_4. × 29,000.

FIGURE 4 A cell that has been starved for 22 hr and then mixed with the opposite mating type for 5 min. The saccules have become swollen throughout much of their length and vesicles (short arrows) appear nearby. A dense body usually lies in the vicinity of these saccules (long arrow). Fixed in OsO_4. × 48,000.

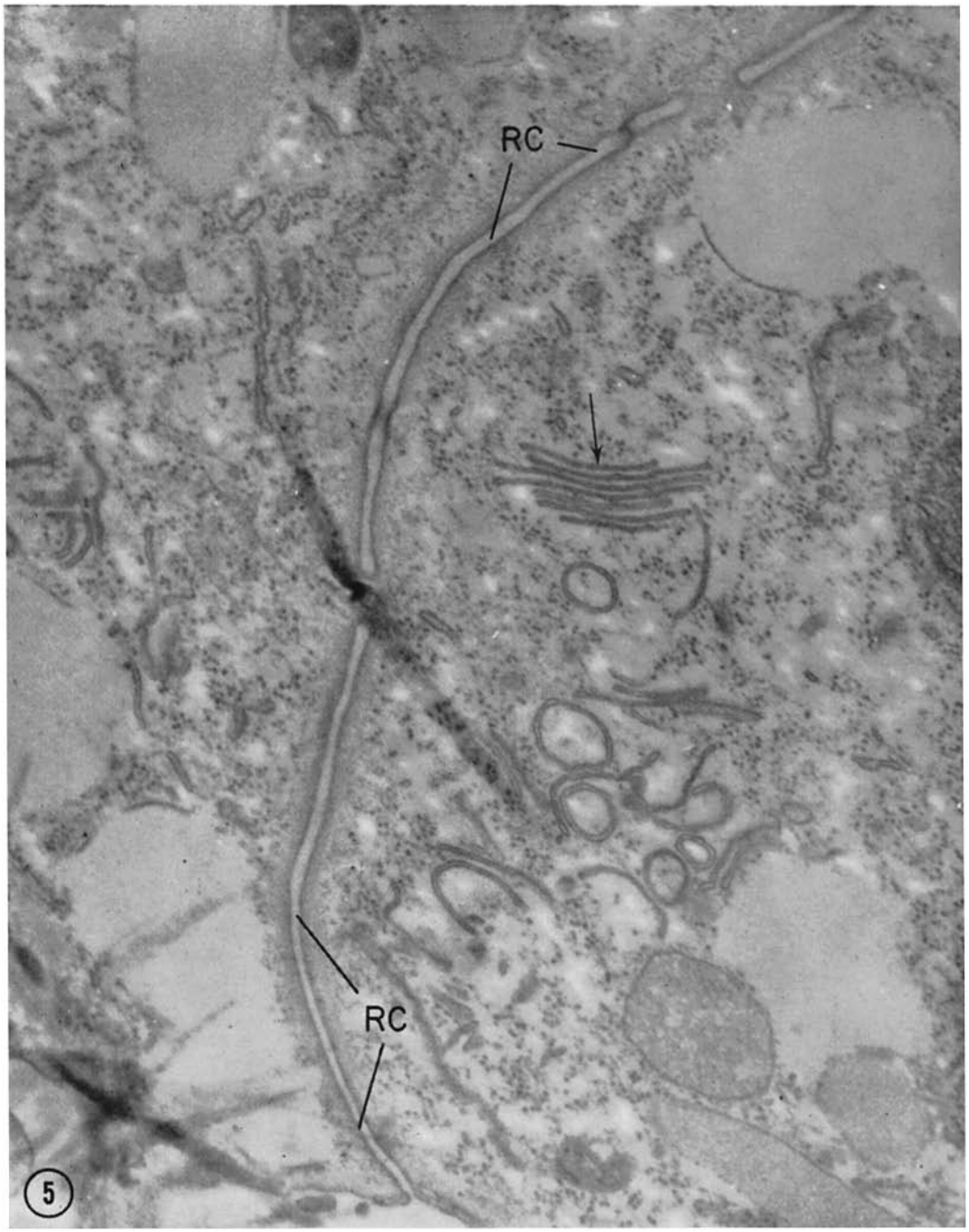


FIGURE 5 This section is taken through the region of contact (*RC*) of mating cells. The cells were mixed for 7 hr and were conjugating for 2–3 hr. Some of the saccules are stacked (arrow), whereas others are not. There are no vesicles and no swollen regions of the saccules, suggesting that no products are being packaged for release into the cytoplasm. Fixed in glutaraldehyde, postfixed in OsO_4. × 42,000.

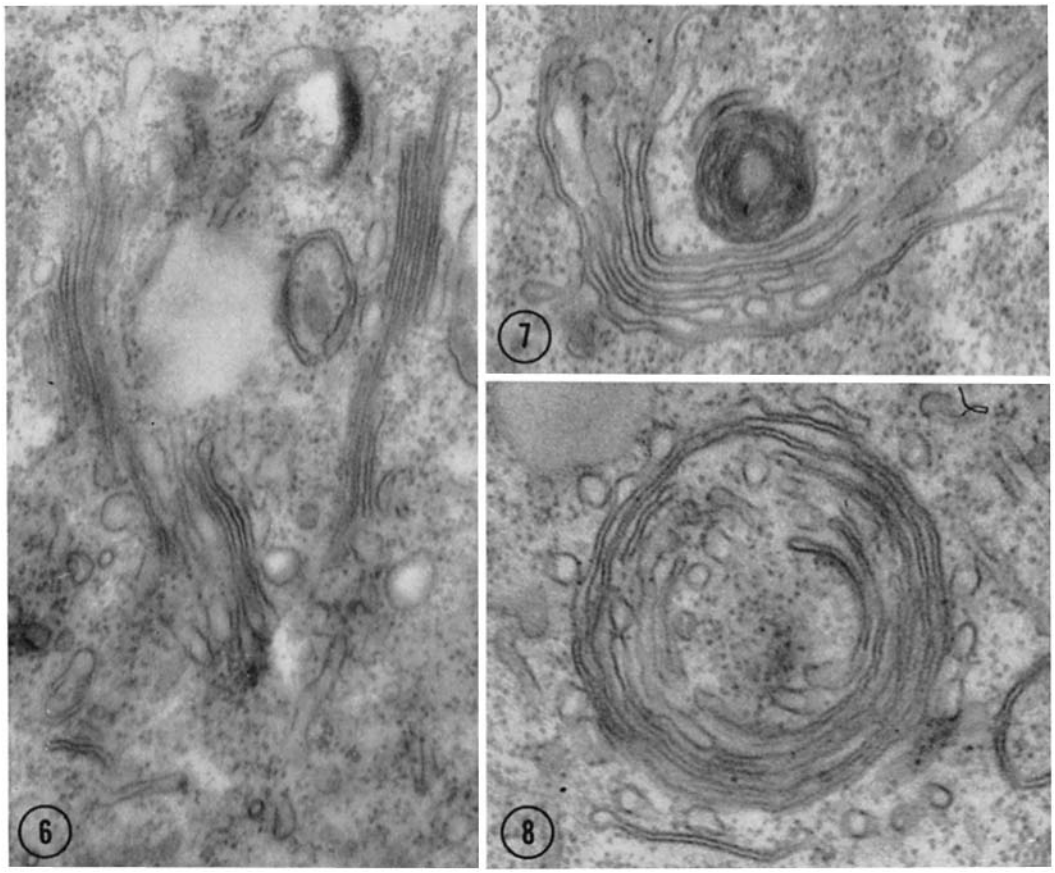


FIGURE 6 This cell was mixed with its mate for 10 hr and probably was conjugating for 7–8 hr. The stacked saccules have swollen ends and numerous vesicles lie in their immediate vicinity. This structure resembles the classical Golgi apparatus. Fixed in OsO_4. × 48,000.

FIGURE 7 This section is from a cell that had been mixed with its mate for 23 hr and is in the last stages of conjugation, or it may be an exconjugant. The saccules possess swollen ends and vesicles seem to be pinching off from them. The dense body containing whorls of membranes is usually associated with the saccules. Fixed in OsO_4. × 55,000.

FIGURE 8 This section is taken from a very recent exconjugant. The Golgi apparatus shows a circular pattern which is often seen. Numerous vesicles are present, suggesting that products are being packaged and released into the cytoplasm. Fixed in OsO_4. × 50,000.

The failure in our laboratory to observe structures resembling the typical Golgi apparatus in *T. pyriformis* was due to the fact that we studied the ultrastructure of only vegetative cells during various phases of growth. The classical Golgi apparatus consisting of stacked, smooth saccules with associated vesicles (2) came to our attention when an ultrastructural study of conjugation was initiated.

Strain E cells (5), as well as several other strains of *T. pyriformis* that we have examined (unpublished), show smooth, flattened, single saccules in the oral region. Their membranes are characteristically thick and dense. Similar saccules have been observed by other workers in a number of ciliates including several species of ophryoscolecids (18), two species of peritrichs, *Epistylis* (8) and *Campanella* (1), *Paramecium* (23), and *Blepharisma* (13). The saccules vary somewhat in shape in these ciliates, but they are always associated with

oral structures. Whether or not they have common origins and functions is impossible to determine from ultrastructural evidence.

Conjugation is induced when ciliates are subjected to a period of starvation (6). During this period, the isolated saccules noted in growing cells appear to become aligned into two or more lamellae. These lamellae are concentrated in the oral region, although they occasionally occur along the anterior macronuclear membrane as well. When opposite mating types are mixed, stacked saccules with terminal swellings and associated small vesicles are observed in the oral region. Their location and structure suggests that they are the same saccules noted above which have become active. When the ciliates are in the presence of the opposite mating type, the Golgi apparatus is apparently stimulated to package some substance contained in the saccules. The vesicles seem to be produced prior to the fusion of the mates, after a period of starvation, and then again midway or near the end of conjugation. The fact that no vesicles are observed for a short period after fusion suggests that no products are being packaged at that time. Since neither the saccules nor the vesicles contain reaction products when treated for the presence of acid phosphatase, it is unlikely that hydrolases are being packaged in the Golgi apparatus described here. At present, we have no information regarding the nature or function of the substance contained in the saccules or vesicles. Its appearance, associated with specific stages in the conjugation cycle, implies that the substance packaged may function in the mating process, perhaps acting as, or activating, receptors (or receptor mechanisms) to the opposite mating type. Or it may be involved in the actual fusion process.

It is well known that the Golgi complex functions as a condensing and packaging station for many secretory products in metazoan cells (19). In protozoa, it also functions as a source of a variety of products. For example, Grimestone (12) postulated that it may function in the production of polysaccharides in *Trichonympha*. Manton and Parke (15) showed that the theca of *Platymonas* was formed by the coalescence of particles derived from the Golgi apparatus. Sommer and Blum (24) demonstrated the presence of acid phosphatase in the Golgi apparatus of *Euglena gracilis*. Daniels (3) supports the view that the Golgi apparatus in amoebae originates from the plasmalemma during pinocytosis and phagocytosis and may transport nitrogenous wastes to the contractile vacuole. In *T. pyriformis*, the Golgi apparatus may be the source of substances essential in mating. Further studies may reveal the function of this organelle.

This investigation was supported by a Research Grant AI 01416-15 from the National Institutes of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service.

We wish to express our appreciation to Mrs. Ann E. Hunt for technical assistance.

Received for publication 11 September 1967, and in revised form 17 October 1967.

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