

THE ULTRASTRUCTURE OF THE PELLICLE COMPLEX OF *EUGLENA GRACILIS*

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ABSTRACT

The pellicle complex of *E. gracilis* is composed of the cell membrane, the ridge and groove with the notch, four fibrils, and the subpellicular ER. The cell membrane is of unit membrane configuration and covers the outside of the cell, the cytostome, the gullet, and the reservoir. The notch of the pellicle complex has always a close topographic relationship to two particular fibrils, as well as the subpellicular ER. The gullet is that region between the reservoir and the cytostome which, in addition to longitudinal fibrils, is surrounded by a single row of circular fibrils. The circumference of the cytostome has twenty large pellicular ridges alternating with small pellicular ridges. Alternating tall and small pellicular ridges cover the entire cell during division.

INTRODUCTION

Despite the fact that *Euglena* has been studied extensively with the electron microscope (1-5), uncertainty still exists regarding the pellicle and its related structures. For example, Gibbs, studying *Euglena gracilis*, and de Haller, studying *Euglena viridis*, suggested that the pellicle consisted of three layers with a combined width of approximately 250 A (1) and 300 A (2), respectively; while Ringo (3), studying *Astasia longa*, indicated that what he refers to as the cell wall is more on the order of 100 A. Moreover, Roth, who studied a system of fibrils in protozoa, did not find such fibrils below the ridges of the pellicle in *Euglena gracilis* (6), but Gibbs (1) described two or three of these in that location. Similarly, Ringo did not find these fibrils in *Astasia longa*, but we have seen them in all of our preparations of the same organism.

This communication presents a detailed study of the "pellicle complex" of *Euglena gracilis*, which was especially warranted because we have shown in the preceding paper that induced acid phosphatase activity is localized in specific regions of the pellicle of *Euglena gracilis*.

MATERIAL AND METHODS

Euglena gracilis, var. *bacillaris*, strain SML-1, were sampled from cultures kept under conditions described earlier (7). The culture medium was decanted after centrifugation at approximately 2,000 RPM for 2 minutes. The loose pellet was resuspended in permanganate (8) or osmium tetroxide with sucrose (9) and fixed for 1 to 2 hours in the cold. The cells were then washed in Ca-free Ringer solution and embedded in Epon or Maraglas. The sections were cut on a standard Porter-Blum ultramicrotome and stained with lead hydroxide for 5 minutes, according to the first procedure of Karnovsky (10), in a nitrogen atmosphere. The sections were viewed with the Hitachi HS 7 or the RCA EMU-3G electron microscopes at an accelerating voltage of 50 kv, with condenser apertures of 250 μ and objective apertures of 30 to 40 μ , at original magnifications between 3,000 and 32,000.

RESULTS

The pellicle of *Euglena gracilis* was composed of ridges and grooves of fairly constant dimensions. At the anterior pole of the cell the ridges converged

and entered the cytostome (Fig. 1), where they diminished in size and virtually disappeared at the entrance of the gullet.

The cell membrane (Fig. 2), a triple-layered structure of unit membrane configuration (11) which covered the ridges and grooves, continued on to cover the gullet, the reservoir, both axonemes, and the single flagellum emerging from the axonemes at the level of the paraflagellar body. In many cells, granular material was seen to cover the cell membrane (Fig. 2).

The pellicular ridges displayed a notch on one side (Fig. 2). Subjacent to the notch were between 3 and 5, but usually 4, fibrils measuring approximately 250 Å in diameter. Two of these were always close together, with one actually touching the cell membrane. It may be for this reason that the latter was sometimes quite obscured (Fig. 2, *TF 3*). The pellicular ridges were rather uniform in size except in the region of the cytostome, where alternating tall and small ridges were observed (Fig. 3), and in dividing cells in which alternating tall and small ridges covered the entire cell (Fig. 4). On 5 suitable cross-sections through the cytostome, 20 tall ridges alternating with an equal number of small ridges were counted. An identical count was made on one such section in an illustration by Wolken (12), even though the author indicated 21 ridges. Some of the small ridges were very shallow, but their existence was always underscored by the invariable presence of usually 4 fibrils (Fig. 4). Pursuing the fibrils farther down into the cytostome, one noted that they extended in pairs in single file along the gullet just beneath the cell membrane (Fig. 5). In addition, the gullet, between the cytostome and the res-

ervoir, was surrounded by a single row of approximately 70 to 80 circular (or helical) fibrils (Figs. 1, 5). In some cells, the circular fibrils were separated from the longitudinal fibrils by a dense filamentous matrix (Fig. 1, 5). More often this matrix was particularly prominent at the gullet-cytostome junction (Fig. 7), similar to comparable accumulations sometimes observed around the kinetosome.

The characteristic tubule of the endoplasmic reticulum was always present throughout the length of each pellicle complex (2, 5, 6). This characteristic association appeared to be heralded in the region of the cytostome by the periodic arrangement of components of the endoplasmic reticulum (Fig. 7), although in this region pellicular ridges cannot, as yet, be distinguished. The tubules of the subpellicular endoplasmic reticulum always had a close topographic relationship with the notch of the pellicle complex, and they also had connections with tubules of adjacent ridges (Fig. 8) as well as with the endoplasmic reticulum deeper in the cell (Fig. 9).

DISCUSSION

The pellicle complex is composed of: (*a*) the cell membrane, (*b*) the ridge and groove with the notch, (*c*) the four fibrils and (*d*) the subpellicular ER. Of these components the notch was of particular interest, because it is here that induced acid phosphatase activity first appears after phosphate withdrawal in *Euglena gracilis* (7). While the notch was lined by an uninterrupted cell membrane which appeared to be no different from that overlying other regions of the pellicle complex, the notch was unique in its close association with two fibrils and with the subpellicular ER. The signifi-

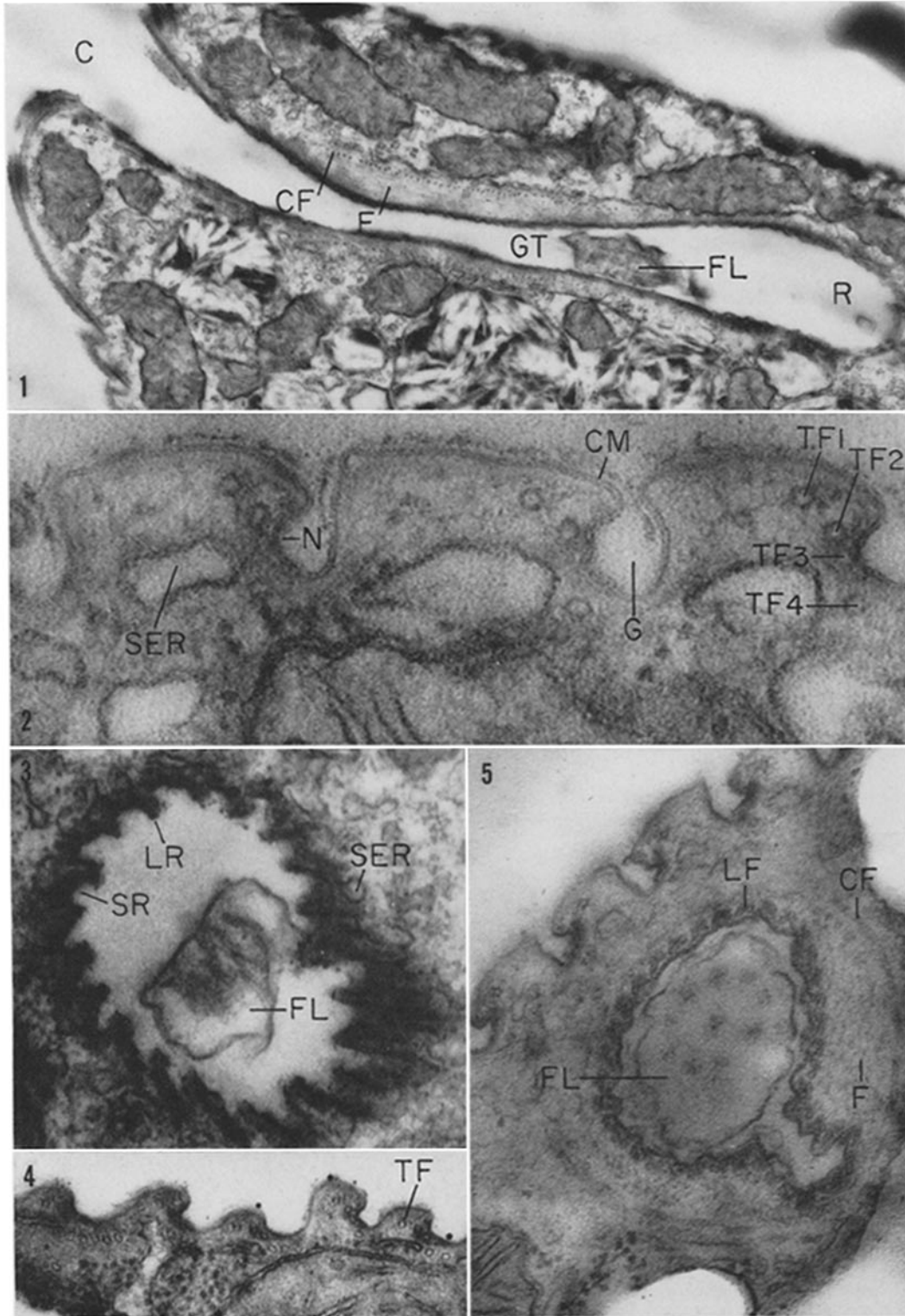
FIGURE 1 Section of cytostome, gullet, and reservoir. *FL*, flagellum; *CF*, circular fibrils; *GT*, gullet; *R*, reservoir; *F*, filaments; *C*, cytostome. Original, $\times 5,600$; final, $\times 17,200$.

FIGURE 2 Pellicle complex. *G*, groove; *CM*, cell membrane; *N*, notch; *SER*, subpellicular ER; *TF 1, 2, 3, 4*, fibrils. Original, $\times 32,000$; final, $\times 128,000$.

FIGURE 3 Transverse section through cytostome (*cf.* Fig. 1). *SER*, subpellicular ER; *FL*, flagellum; *SR*, small pellicular ridge; *LR*, large pellicular ridge. Original, $\times 5,600$, final, $\times 38,000$.

FIGURE 4 Alternating small and large pellicle complexes of a cell in division. *TF*, fibrils. Original, $\times 11,000$; final, $\times 40,000$.

FIGURE 5 Transverse section through gullet. *CF*, circular fibril; *LF*, longitudinal fibril; *F*, filamentous matrix; *FL*, flagellum. Original, $\times 11,000$; final, $\times 57,200$.



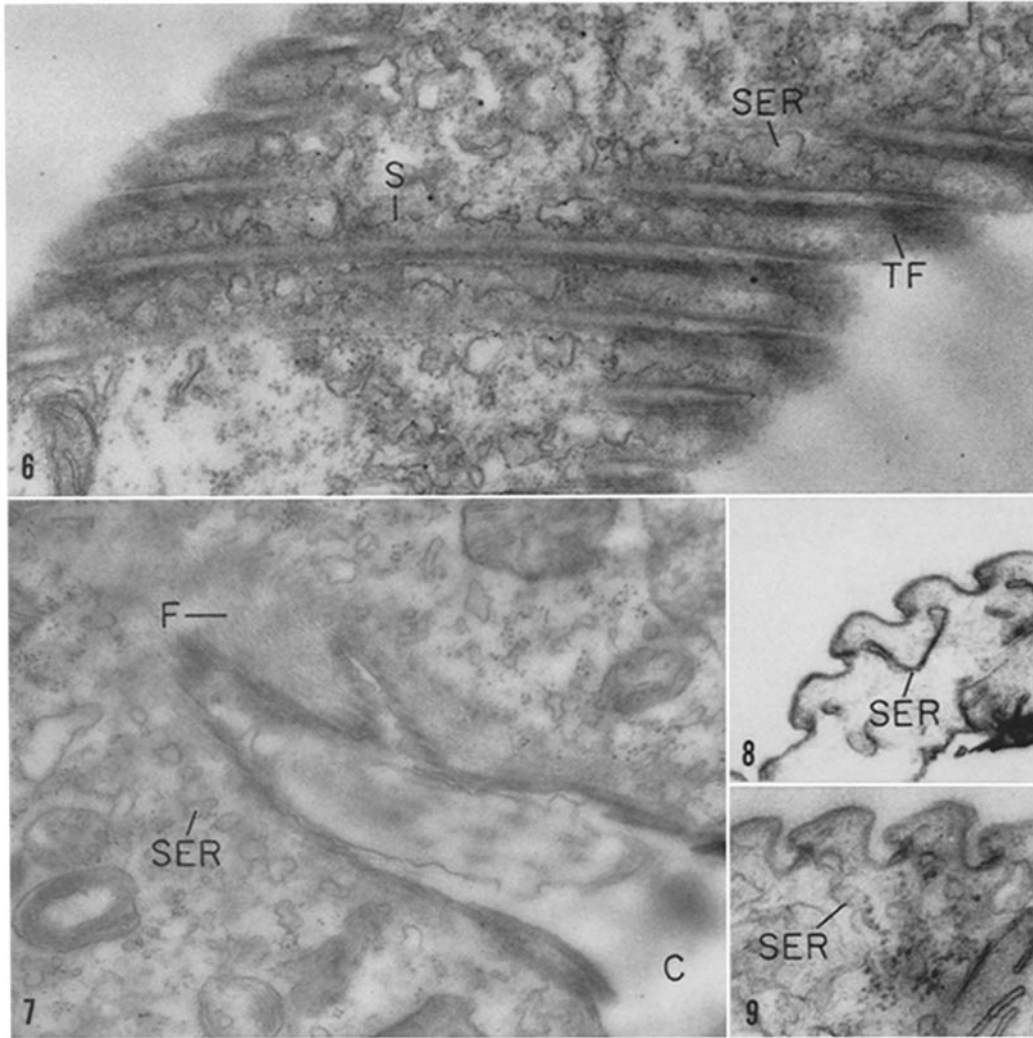


FIGURE 6 Longitudinal section through the pellicle. *TF*, fibrils; *SER*, subpellicular ER. Note short cross-striations (*S*) on that side of the groove which is opposite the notch, *i.e.* opposite to where the fibrils are located. Original, $\times 10,000$; final, $\times 38,000$.

FIGURE 7 Longitudinal sections through the cytotome region. *SER*, subpellicular ER; *F*, filamentous matrix. Original, $\times 11,000$; final, $\times 35,200$.

FIGURE 8 Cell fixed in permanganate. A connection is seen between the subpellicular ER of two adjacent ridges. Original, $\times 10,000$; final, $\times 56,800$.

FIGURE 9 Cross-section of pellicle. *SER*, subpellicular ER, is continuous with the ER deeper within the cell. Original, $\times 11,000$; final, $\times 44,000$.

cance of these anatomical findings for the localization of acid phosphatase in this region in *Euglena gracilis* is quite obscure, particularly since no induced acid phosphatase was demonstrable in the

pellicle of *Astasia longa*, although the pellicle complex of the latter is very similar to that of *Euglena gracilis*.

Of great interest was the observation that in the

area of the cytostome there were always twenty tall ridges alternating with shallow ridges. The observation that this arrangement of alternating ridges occurred outside the cytostome, but only in a dividing cell, may be of great importance for understanding the mechanism of cell division in the *Euglenidae*. The fact that sometimes four fibrils were observed underlying just barely elevated ridges, or, in some cases, just a flat cell membrane, suggests that the fibrils, among other things, may have an influence on the formation of the pellicular ridges. Ridges without fibrils were never seen. No

information is available concerning how the fibrils are formed. However, on the basis of the observations regarding the filamentous matrix surrounding the gullet (Figs. 2, 7, 10), one might speculate that it is from this matrix that the fibrils arise.

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