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Periplast Structure of the Cryptomonad Flagellate *Hemiselmis brunnescens*

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Summary

The periplast of *Hemiselmis brunnescens* BUTCHER is a complex cell covering comprised of the plasma membrane (PM) sandwiched between a surface periplast component (SPC) and an inner periplast component (IPC). The SPC is revealed by deep-etching, and consists of hexagonal plates composed of tripartite subunits that appear to self-assemble into a crystalline layer with a hexagonal symmetry. Small scales (termed fibrillar scales) accumulate on the crystalline plates during cell growth, eventually forming a "carpet" that itself may appear crystalline when fully formed. Heptagonal "rosette scales" are occasionally observed on the surface as well. The position of the crystalline plates is precisely mirrored by both the E and P fracture faces of the PM. The plate proper is underlain by membrane with a high concentration of intramembrane particles (IMPs) while the bands of membrane underlying the plate borders lack IMPs. Access of subunits and fibrillar scales to the cell surface following initial plate formation appears to be at the plate boundaries. This study suggests that cryptomonad flagellates may provide model systems for studying the self-assembly of cell surface components, and for relating membrane structure to function, as evidence suggests a major role for the PM in mediating periplast assembly and development.

Keywords: Cell surface; *Cryptophyceae;* Freeze fracture/etch; Periplast; Scales; Self-assembly.

1. Introduction

Cryptomonad flagellates are common in most aquatic environments and display a number of unique features. Cells possess a characteristic asymmetry with two flagella arising anterio-laterally from a furrow or vestibulum/gullet region on the ventral surface. Cytoskeletal microtubules and rigid wall components are absent, and a structure referred to as the periplast is responsible for the maintenance of cell shape (GANTT 1971). The periplast consists of a surface periplast component (SPC) appressed to the PM surface and an inner periplast component (IPC) associated with the cytoplasmic surface of the PM. The structure, position and function of both periplast components may vary considerably between different cryptomonad species and appears to be of taxonomic significance (SANTORE 1977, 1982a, b).

Most investigators have used scanning electron microscopy and thin sectioning techniques to describe periplast structure, although GANTT (1971) and FAUST (1974) were successful in isolating components of the IPC from *Chroomonas* sp. and *Cryptomonas ovata* for structural analysis using negative stained whole mounts. The IPC is found in several configurations, being tightly appressed to the inner PM surface (SANTORE 1977, 1982a), forming a distinct layer of hexagonal, rectangular or polygonal plates that are only associated with the PM at their edges (*e.g.*, GANTT 1971, FAUST 1974, SANTORE 1977, 1982b), or producing a sheet beneath the PM (BRETT and WETHERBEE 1986). Some workers have observed the impressions of inner and/or outer plates in fracture faces of the PM (GANTT 1971, HAUSMANN and WALZ 1979) as well as the substructure of the PM and its relationship to the plate layers (HAUSMANN and WALZ 1979) and underlying ejectisomes (GRIM and STAEHELIN 1984). However, the precise structure of periplast components, particularly the cell surface layer, is largely unknown. We are employing deep-etch to describe the detailed structure

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of the SPC in various cryptomonads (BRETT and WETHERBEE 1986), and this paper describes the SPC of *H. brunnescens* and its relationship to the PM and IPC during development. The complex cell surface appears mostly crystalline, with hexagonal plates and an associated scale layer self-assembling from components deposited on the surface. We believe the PM mediates the assembly and development of the SPC and perhaps the IPC as well.

2. Materials and Methods

H. brunnescens was obtained from the Cambridge culture collection (CCAP 984/2) and grown in f/2 enriched seawater (GUILLARD and RYTHER 1962) at 16 °C with a light/dark cycle of 16/8. The light micrograph was taken using Nomarski optics on a Zeiss photomicroscope III. The scanning electron micrograph was taken on a Philips 505 SEM following fixation in 1% Lugol's iodine solution, postfixation in 1% OsO_4 solution, dehydration in EtOH and gold coating in a sputter coater.

For transmission EM, cells were concentrated by centrifugation and fixed for 1 hour (occasionally for two hours) in 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.5) osmotically balanced with 0.4 M sucrose. Cells were gradually brought into pure buffer (4 changes), postfixed in 1% OsO_4 in buffer, dehydrated in a graded EtOH series and embedded in Spurr's low viscosity medium. Blocks were thin-sectioned with a diamond knife, stained and viewed on a Siemens 102 electron microscope.

Material for freeze fracture and etch was unfixed, frozen in Freon 22, etched for varying times (normally 2 minutes) and shadowed. Unidirectional shadowing was at 45° while rotary shadowing (50 rpm) was from an elevation of 22.5°. Replicas were cleaned either in bleach or a 50% solution of chromerge and mounted on 300 mesh grids for viewing. With micrographs of rotary shadowed replicas, we have printed the positive.

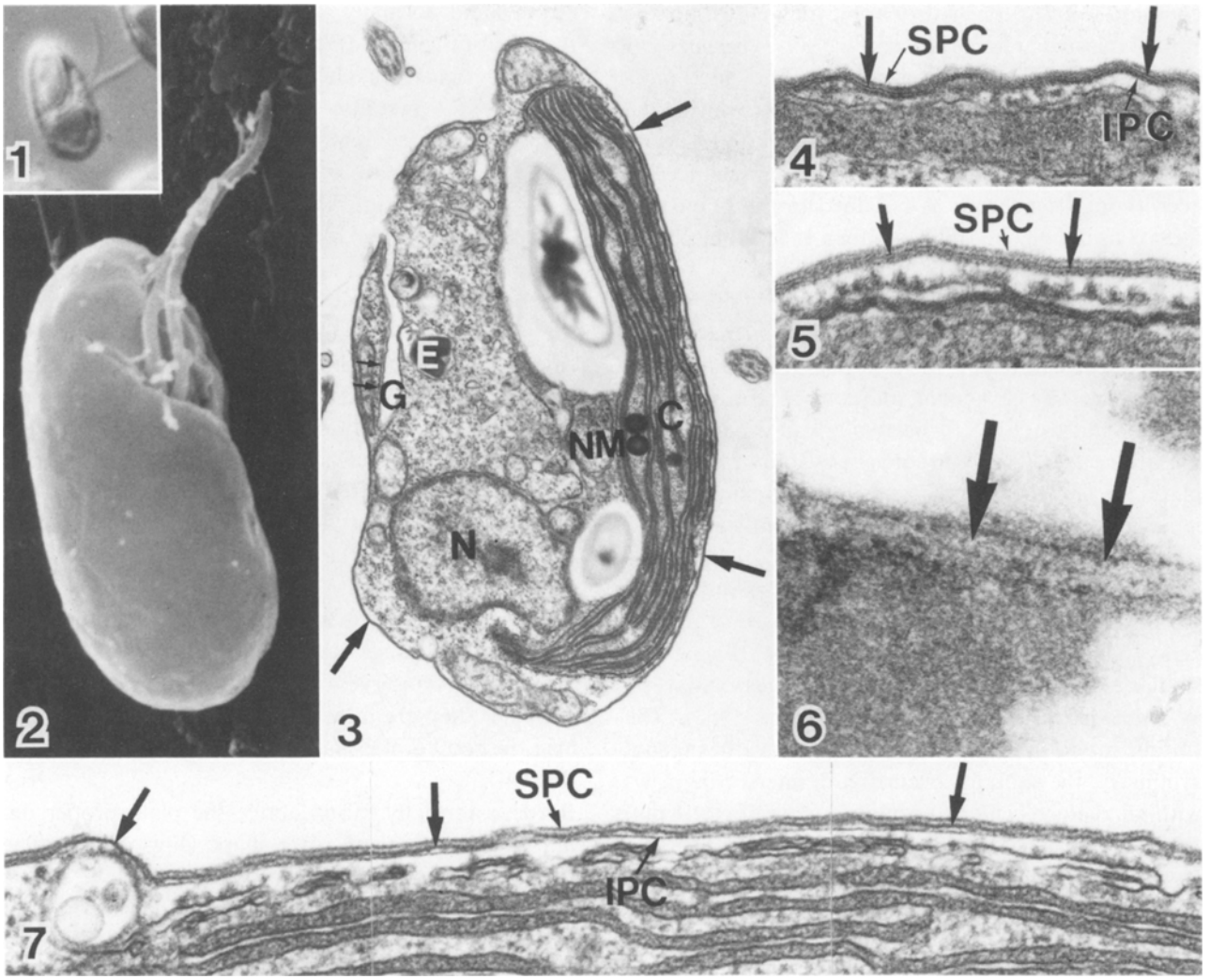
3. Results

H. brunnescens is a small (ca. 8 μm in length), biflagellate, asymmetric unicell dorsoventrally flattened with the two flagella emerging subapically from a depression or vestibulum (Figs. 1 and 2). Various fine-structural features characterizing the cryptomonads are observed in *H. brunnescens* (SANTORE 1977, 1982a, PENNICK 1982). Chloroplasts possess loosely paired thylakoids containing an electron dense phycobiliprotein (Figs. 3 and 7) within the intrathylakoid spaces. Other distinguishing features include the nucleomorph (Fig. 3), a double membrane-bound organelle containing DNA (LUDWIG and GIBBS 1985), and a complex periplast that encloses the cell. The periplast is interrupted only at the vestibulum/gullet region where the PM is not associated with the normal periplast components (Figs. 3, 14, and 21). The envelope encasing the two flagella, which is continuous with the PM at the vestibulum, comprises a third region of the cell surface and is characterized by attached hairs and scales (PENNICK 1981, SANTORE 1983). In this report we are mostly concerned with the structure and development of the SPC and its relationship to the substructure of the underlying PM.

Light and scanning electron microscope observations of the *H. brunnescens* periplast are made difficult by the small and delicate nature of the cells and by the absence of the highly organized rows of ejectisomes and prominant plate areas seen in larger cryptomonad genera (*e.g.*, certain *Cryptomonas* spp. and *Chroomonas* spp.), the cell surface appearing smooth and featureless (Figs. 1 and 2). In contrast, thin sections reveal a number of structural features (Figs. 3–7): the periplast consisting of the PM sandwiched by two distinct layers, the SPC and IPC (Figs. 4–7), that differ from one another in structure, thickness, flexibility and apparent function. The micromorphology of both layers may vary according to the stage in development or the manner of preparation for electron microscopy. Thin sections show the outer plate layer, which is generally more electron dence at its surface, to be closely appressed to the PM surface, following the contours of the PM exactly. Although divided into distinct hexagonal plates (see below), the plate structure is not readily recognized in thin sections. However, the boundaries between plates can be observed in glancing section (Fig. 6).

The IPC is thinner than the SPC and therefore more difficult to discern in thin section. The IPC may appear directly appressed to the PM (Figs. 4 and 5), closely following the contours of both the PM and outer plate layer, or occasionally detached from the under-surface of the PM (Fig. 7), although retaining its cohesiveness. The extent of separation appears to relate to the fixation conditions, and is most frequent where the PM has become slightly convoluted. These small convolutions in the PM and the resulting separation of the IPC are not observed in replicas of unfixed, freeze-fractured cells and are believed to be fixation artifacts. However, since this inner layer readily separates from the PM while retaining its integrity, it is relatively more rigid than the thicker SPC.

Gaps, visible in both periplast layers in thin sections, are not apparent in deep-etches of the SPC. Gaps are most obvious where ejectisomes (ejectile organelles found in the cryptomonads) underlie the PM. Ejectisomes, very common in some species, are occasionally observed in *H. brunnescens* lining the gullet (Fig. 21) or lying just beneath the cell surface where the overlying



Figs. 1 and 2. Light and scanning electron micrographs of *H. brunnescens*. The cell surface appears smooth and non-distinctive. Fig. 1 × 2,500; Fig. 2 × 9,500

Fig. 3. Thin section showing the gullet (*G*) in longitudinal section. Note the surrounding periplast (large arrows) that appears as a thick line at this magnification and the gullet-associated material lining one side of the gullet only (small arrows). Other cellular components include the chloroplast (*C*) with paired thylakoids, nucleomorph (*NM*), nucleus (*N*) and a single ejectisome (*E*). × 17,000

Fig. 4. Cell fixed during the period of active division and growth. Both the SPC and IPC are electron dense and tightly appressed to the PM surface. The SPC is thicker than the PM (arrows) while the IPC is thinner. × 75,000

Fig. 5. Cells fixed during the stage of periplast development. the SPC is thicker than the PM (arrows) and its surface is electron dense. The IPC is tightly appressed to the underface of the PM. × 100,000

Fig. 6. Glancing section revealing the boundary (arrows) between plates of the SPC. × 102,000

Fig. 7. Cell from a culture in the stationary phase fixed for 2 hours. The SPC is more than twice the thickness of the PM (arrows) but follows its contours. The IPC appears about half the thickness of the PM when separated from it. Note the electron dense intrathylakoid space which contains the phycobilin pigments. An empty ejectisome vesicle appears beneath the PM where the SPC and IPC are missing, although there are no other obvious gaps that could correspond to plate borders. × 84,000

plates abut. Vesicles may also occur here beneath the PM, although when empty,they imply that an ejectisome has been extruded (Fig. 7). In some early stages of development, gaps may also correspond to the junction between external plates. Gaps may occur in the SPC only, or in both the SPC and IPC. Distortions due to fixation account for many of the variable images obtained, and gaps may not be as common as suggested by other workers.

The most accurate picture of the surface morphology of cryptomonad flagellates comes from freeze-etching of intact, unfixed cells. The only fracture in the periplast

complex follows the hydrophobic region within the PM. Extensive areas of the surface of *H. brunnescens* are then exposed by deep-etching. The micromorphology of the SPC has been shown to vary during the cell cycle. However, it is somtimes difficult to associate stages in periplast development with particular cells, even though synchrony is obtained to the point that most cells divide once daily within a 1–1.5 hour period (cytokinesis takes 3–5 minutes per cell).

Electron micrographs of replicas prepared during the period of maximum division show cells to possess large, plate-like structures on their surface (Figs. 8 and 10). Plates, generally hexagonal, may vary in size and shape over the surface (Fig. 16), particularly at the apical and posterior ends of a cell. In some cases four or five-sided plates may reflect an early stage in development, and it is possible that these plates are subsequently altered to six-sided.

Upon close inspection, the plates, which correspond to the SPC seen in thin section, are composed of regular arrays of macromolecules or subunits (Figs. 8–11) similar to the outer crystalline surface layers (S-layers) in many bacteria (SLEYTR and MESSNER 1983). The surface monolayer of *H. brunnescens* has a hexagonal symmetry, the subunits occurring in intersecting rows with an average center-to-center spacing of ca. 18 nm. With unidirectional shadowing the crystalline surface layer looks complete, with few, if any, irregularities within the plate areas (Figs. 8 and 9). However, rotary shadowing reveals the crystalline layer in more detail (Figs. 10 and 11), each subunit having a tripartite, clover leaf appearance. Irregularities occur in the crystalline pattern as well as small, possibly artifactual, connections between adjacent subunits.

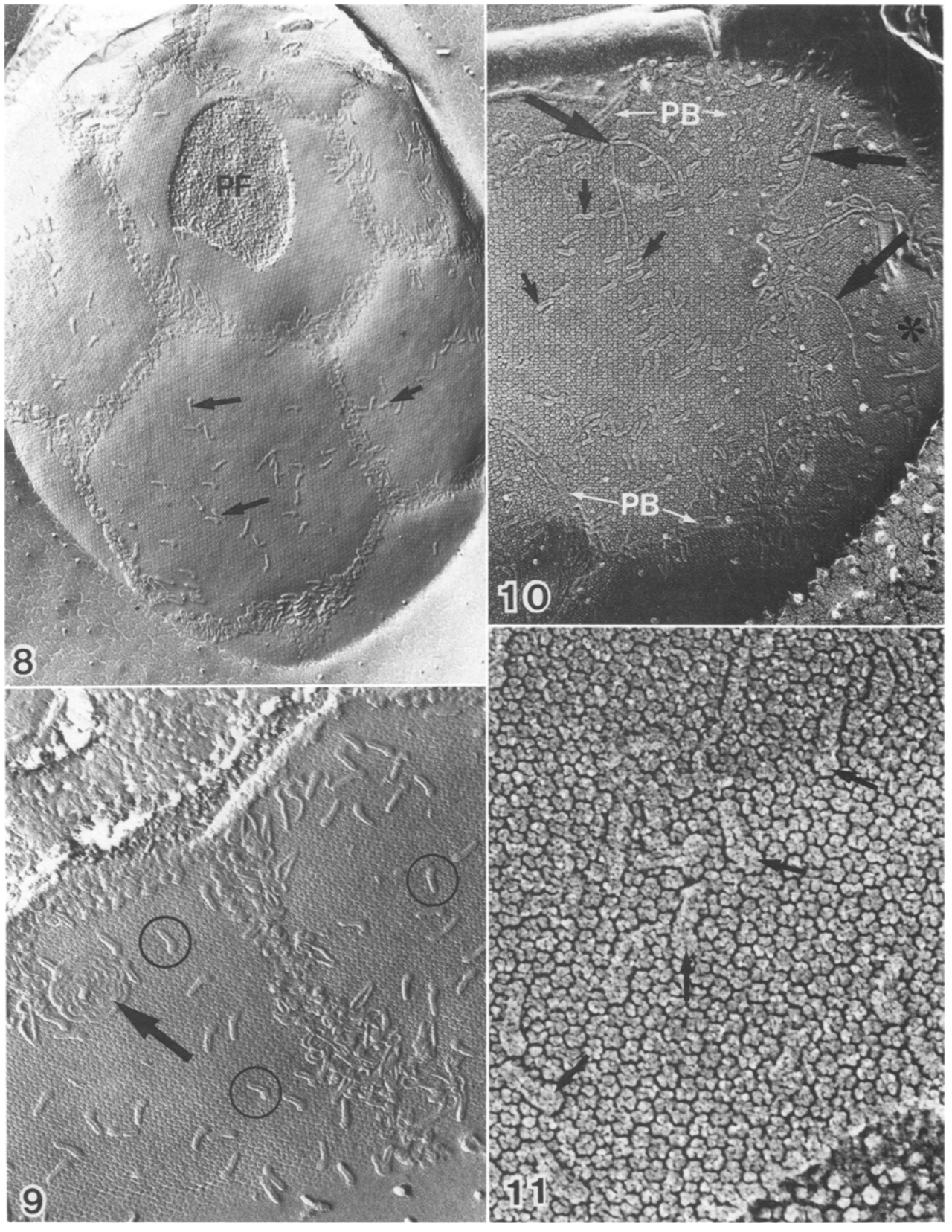
Additional wall components are associated with the crystalline plates, and their concentration varies during the cell cycle. The most conspicuous are "fibrillar scales" that accumulate on the crystalline subunits (Figs. 8–16). Uniform in structure and size, they consist of a globular head (ca. 22 nm in diameter) with a tail comprised of two fibrils slightly unequal in length (ca. 40 nm), and normally attached to one another along their entire length (Figs. 9 and 11–13). While the globular heads are always positioned over a single subunit of the crystalline plate, tail fibrils lying down on it may be oriented in any direction. Fibrillar scales are scarce on the surface of many cells just following the division period (*e.g.*, Fig. 8), apparently accumulating during subsequent growth (Figs. 12–16). During much of the cell cycle, or in aged cultures, scales completely cover the crystalline surface of the outer plates, giving the surface the appearance of a thick shag carpet (Figs. 15 and 16) which, when etched, may also appear crystalline if the scale tails are oriented in a uniform direction. Presumably this surface image reflects the underlying hexagonal symmetry of the surface subunits. The origin of the fibrillar scales is unclear.

An additional fibrillar surface component is occasionally observed (Fig. 10) and ranges from 21 to 25 nm in width and varies considerably in length. Heptagonal scales (termed "rosette scales") characteristic of several cryptomonads (*e.g.*, BRETT and WETHERBEE 1986) are occasionally observed on *H. brunnescens* (Fig. 9), although they are more common on the flagella and near the point of flagellar attachment in the vestibulum (Fig. 14).

Borders normally raised above the plate proper delineate the hexagonal plate shape (Figs. 12–14). The structure and position of these borders change during the cell cycle; these changes appear necessary for two reasons: first, to allow for changes in plate size and shape during the cell cycle, and secondly, the border material may participate in the formation and assembly of the fibrillar scale layer. The exact developmental sequence is not yet known, although the plate boundaries appear to be established during the formation, or reassembly, of the crystalline plates during cell division and subsequent growth. The structure of the material comprising the plate borders (where growth may be occurring) is highly variable and hard to distinguish in early stages (Figs. 8 and 9). The most common image, probably from fully formed plates, is that of a bi- or

Figs. 8 and 9. Replica of cells freeze-fractured and deep-etched (unidirectional shadowing) during the period of cell division and growth. Fig. 8 illustrates the P fracture face (*PF*) plus the crystalline, hexagonal plates that make up the SPC. Scattered fibrillar scales (arrows) are present on the plate proper, but are concentrated near the plate borders. Fig. 9 is a high magnification micrograph in the same stage. A rosette scale (arrow) is seen on the plate surface and fibrillar scales (circled) are concentrated (forming?) at the plate borders. Fig. 8 × 45,000; Fig. 9. × 90,000

Figs. 10 and 11. Replicas of cells taken at the same stage of development as those seen in Figs. 8 and 9, but rotary shadowed to reveal the offset rows of tripartite subunits that make up the crystalline plates. Fibrillar scales (small arrows) with their heads positioned over a single subunit are scattered over the surface, and a second, longer fibrillar component (large arrows) is seen in Fig. 10. Plate borders (*PB*) are also observed. Note that the edges of the replica (asterisk) in Fig. 10, which are perpendicular to the platinum source during rotary shadowing, have the same image as the unidirectionally shadowed plates seen in Figs. 8 and 9. Faults in the lattice and small connections between the subunits (artifacts?) are observed in Fig. 11. Fig. 10 × 60,000; Fig. 11 × 250,000



Figs. 8–11

tripartite band of segmented material raised above the surface of the crystalline monolayer (Figs. 12–14). This border material often appears associated with fibrillar scales (Figs. 12–14). As the overlying carpet develops, the plate borders can still be discerned as raised areas (Fig. 16). Fibrillar scales on the plate proper are always observed fully formed. The segments that comprise the plate border material (Figs. 12 and 13) are similar in size and shape to the components of the fibrillar scales. As the boundary layers have a highly variable structure (*e.g.*, segments are often missing from the border material), the fibrillar scales may assemble at the plate boundaries and then deploy across the plate surface.

Fracture faces of the PM are shown in Figs. 17–20. The most striking and exact correlation is between the position of the overlying plates and the E and P leafs of the PM. This image is caused by the impression of the outer periplast plates on the PM and the distribution of IMPs, generally missing in the regions of membrane corresponding to the overlying plate borders (Figs. 17–19), although the distribution of IMPs may vary, presumably during development (Fig. 20). The inner leaf of membrane also appears slightly indented towards the cytoplasm at border regions as seen in the P-fracture face (Fig. 17 and 18). The concentration of IMPs is high in other regions of the PM, particularly in the P-face (Fig. 18), although large numbers of IMPs also separate with the outer leaf of the PM (Figs. 19 and 20). The IMPs often appear in rows, and when considering the large number of IMPs that separate with each leaf, their concentration in the intact membrane must be extremely dense, and may even be crystalline. The position of underlying ejectisomes is revealed in the P-face by 2 curved rows of IMPs (inset, Fig. 18).

The tubular gullet arises at a small vestibulum located at the site of flagellar insertion (Figs. 2, 3, 21, 23, and 24). The SPC is discontinuous in the region of the vestibulum (Figs. 14 and 21). In thin sections, this area appears devoid of surface layers (Fig. 21), though deep-etches reveal a fibrillar component, occasionally rosette scales (Fig. 14), and rarely an underlying crystalline layer is exposed and made up of triangular subunits (Fig. 14). It is assumed that these layers are not preserved by the normal fixation procedures used prior to thin sectioning. When observed, etched surfaces of the gullet often appear devoid of material entirely.

The transition region between the normal surface periplast and the vestibulum is seen in fracture faces of the PM (Figs. 23 and 24) where the impression of surface plates ends and there is a decrease in the number of IMPs. The rough appearance of the P face of the PM in the vestibulum may be related to the fusion site of the Golgi and/or contractile vacuole. Although the gullet may be devoid of obvious surface components, a thin layer of gullet-associated material underlies that portion of the gullet membrane nearest the cell surface and away from the main body of the cytoplasm (Figs. 3 and 21). This layer runs parallel to the membrane and electron dense particles are periodically spaced between the two (inset, Fig. 21). While scarce in the P-face of the gullet membrane, a crystalline array of IMPs (Fig. 22) (rectangular symmetry) is seen along that surface of the tubular membrane that corresponds to the overlying, electron dense layer. This band of material may be structural, supporting and positioning the gullet within the cell, and the IMP lattice may reflect the surface attachment sites between this material and the gullet membrane.

4. Discussion

Surface layers have been studied extensively in many algal classes, most notably the surface-associated scale layers found on flagellates from the *Prymnesiophyceae, Chrysophyceae*, and *Prasinophyceae* (for review see

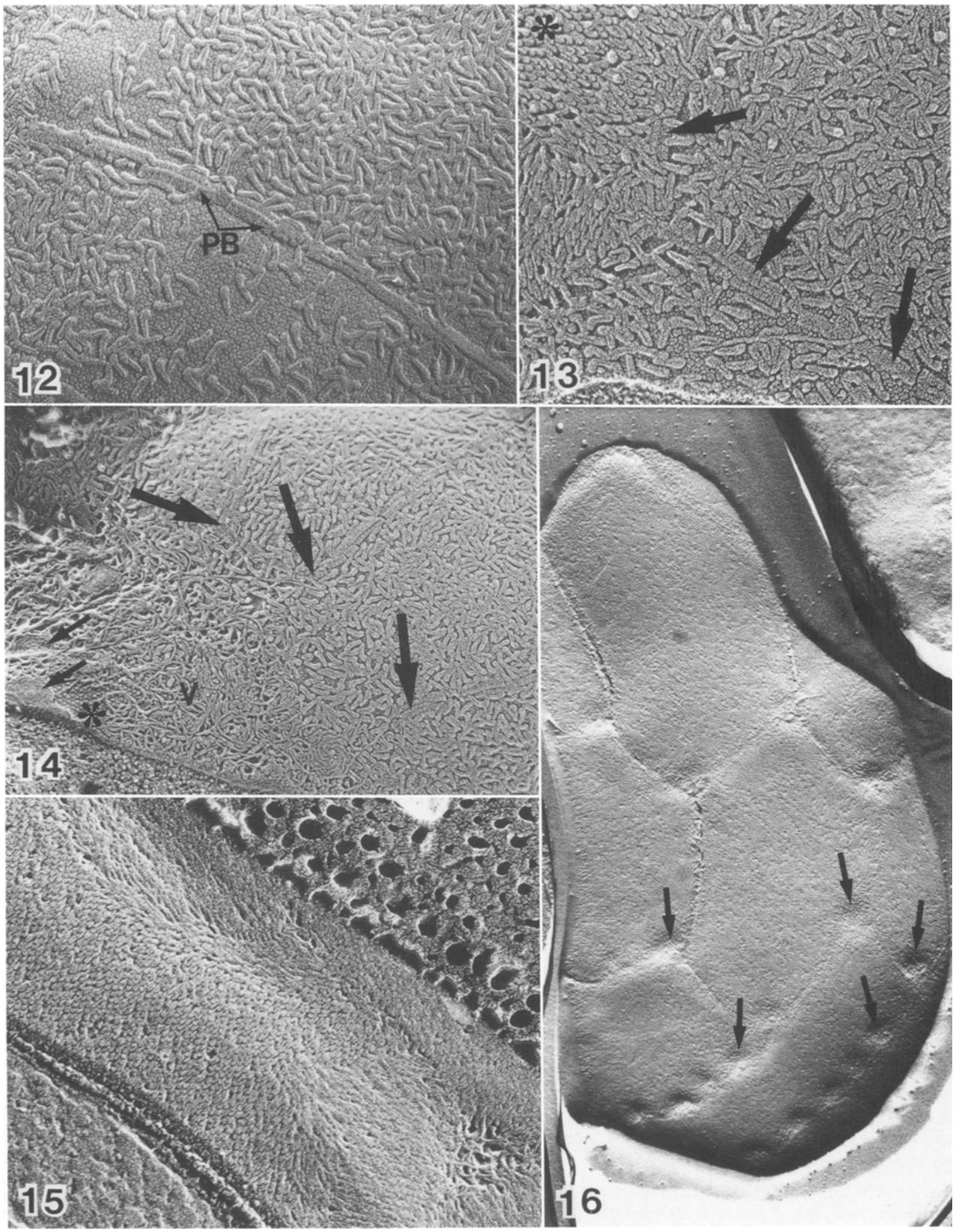
Figs. 12–16. Deep-etches of the SPC, taken several hours after cell division

Fig. 12. SPC of a cell during "carpet" formation. The plate border (*PB*) is raised above the crystalline monolayer. Border material varies in structure but is generally segmented with a few segments absent along its length. Fibrillar scales are plentiful and fully formed (*i.e.*, no obvious stages in the formation of these scales are observed on the plate proper). × 90,000

Fig. 13. Rotary shadowed. The concentration of fibrillar scales is high several hours after the period of division. The segmented plate borders are observed (arrows) and the concentration of fibrillar scales in one region of the surface (asterisk) is high enough to give the appearance of a carpet. × 90,000

Fig. 14. Deep-etch in that region of the surface where the normal SPCs end and the vestibulum (*V*) begins. Plate borders end (large arrows) and fibrillar scales are absent from the vestibulum surface, which is characterized by a curved fibrillar component and the occasional rosette scale (small arrows). A crystalline layer with triangular subunits appears disguised by the fibrils (asterisk). × 45,000

Figs. 15 and 16. Fully formed "carpet" resulting from a high concentration of fibrillar scales. If the "tails" of the fibrillar scales fall in the same direction, they have a crystalline pattern with hexagonal symmetry. Fig. 15. is rotary shadowed and Fig. 16 unindirectionaly shadowed. The depressions seen at plate borders in Fig. 16 (arrows) represent the location of ejectisome vesicles. Fig. 15 × 75,000; Fig. 16 × 40,000



Figs. 12–16

ROMANOVICZ 1981). The SPC of *H. brunnescens*, as well as several other cryptomonad species we have studied (HILL and WETHERBEE 1986, BRETT and WETHERBEE 1986, unpublished results), is not conspicuous using normal methods, but when examined by deep-etch is shown to be highly structured. The surface microarchitecture appears to result from self-assembly, a mechanism of wall formation employed by many bacteria, but only reported in a few algal cells. Fractures through the PM show a distinct correlation between membrane substructure and the position and development of the outer crystalline plates. A role for the PM in mediating the assembly and deployment of the SPC may be inferred, as the plates and scales do not appear to be pre-packaged in the endomembrane system (*e.g.*, the Golgi or endoplasmic reticulum) and secreted as discrete wall units.

The self-assembly of crystalline wall layers has been described for a number of organisms, particularly the crystalline surface layers (S-layers) of some bacteria and cyanobacteria (for review see SLEYTR and MESSNER 1983). Although attached to the outer surface of the wall in many species, the S-layers of some bacteria (*e.g.*, the archaebacteria) are attached directly to the PM. Crystalline wall layers have also been found on eukaryotic cells. The walls of the green alga *Chlamydomonas* contain glycoprotein subunits that self-assemble to form a crystalline component over an amorphous inner wall layer (CATT *et al.* 1976). A spectacular example of self-assembly occurs in the green alga *Scenedesmus* (STAEHELIN and PICKETT-HEAPS 1975), whose highly ornamented walls include a complex of spikelets enclosed in a sheath of hexagonally packed subunits.

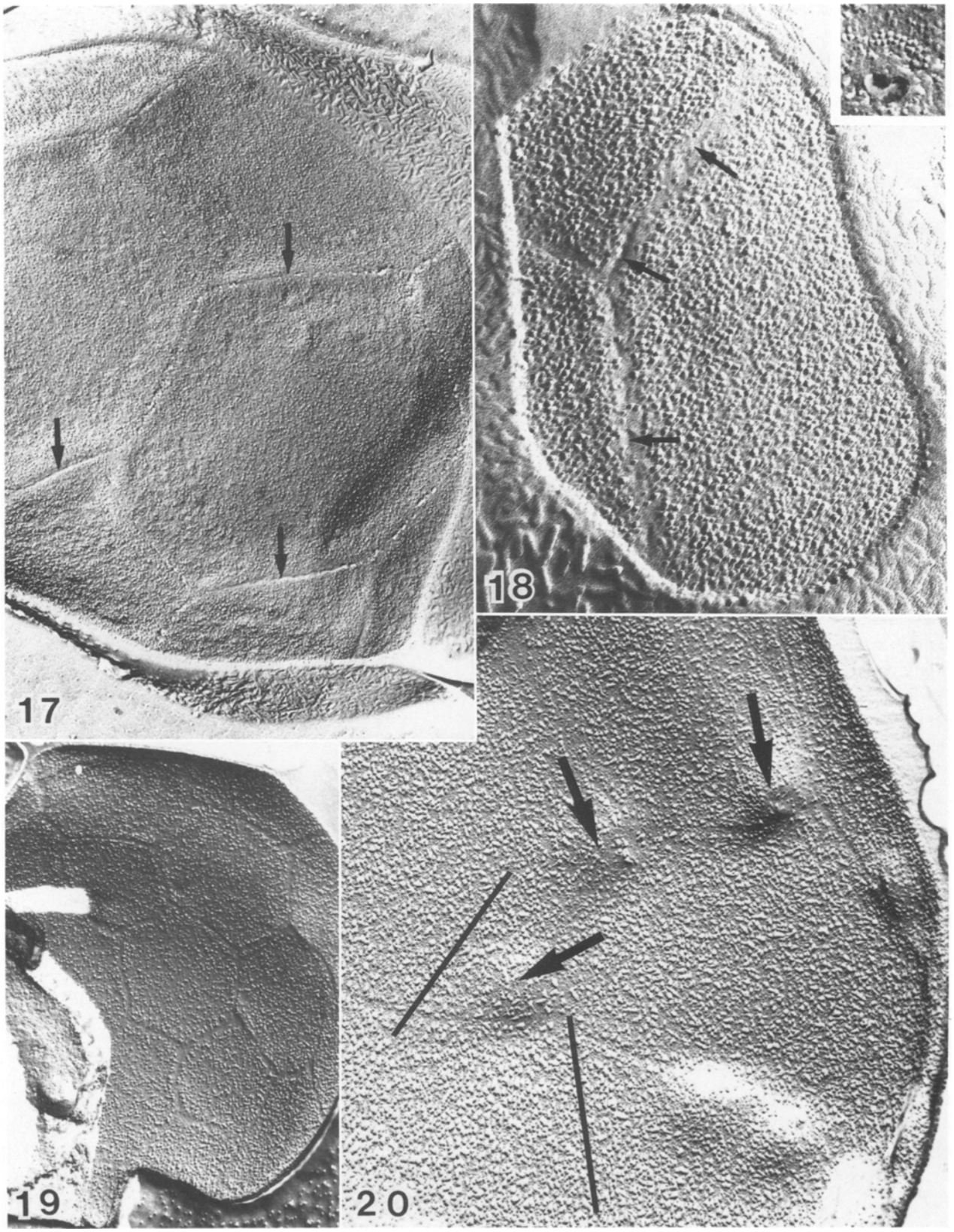
Previous studies of *H. brunnescens* (SANTORE 1977, 1982 a) have not employed techniques that would allow description of the precise structure of the periplast. Reports that it consists of offset rows of rectangular plates can now be discounted, as replicas prepared by deep-etching show crystalline, hexagonal plates with an associated scale layer. The crystalline layer appears similar to many bacterial S-layers, although the architecture of the periplast is considerably more complex. In bacteria the subunits are distributed so as to completely cover the cell (SLEYTR and MESSNER 1983), while in *H. brunnescens* the vestibulum and gullet are structurally and functionally distinct and devoid of normal SPCs. In addition, the crystalline array of *H. brunnescens* has a tertiary organization, being subdivided into distinctly bordered plates and providing a surface for the development and deployment of an outer scale layer.

In bacteria, new subunits appear on the surface at division sites, first forming small crystallites and then rearranging themselves into large areas of uniform patterns. Subunits are reportedly mobile in the plane of the surface, but become comparatively stable once they are in position in the regular arrays (SLEYTR and MESSNER 1983). The mechanism of subunit formation, secretion and subsequent assembly on the surface is not known for *H. brunnescens*. Cells etched during active division and while growing have a high percentage of surfaces displaying the crystalline surface plates (*e.g.*,Fig. 8). Only a few scattered fibrillar scales are seen on these plates, although cell surfaces with a developed "carpet" are also observed. This observation suggests one of two possibilities: either the fibrillar scales are dislodged prior to or during division and growth, or they only remain on that portion of the periplast retained from the parent cell. In the latter event, fibrillar scales would then assemble on the newly formed regions of the periplast in daughter cells. However, as we have not observed an etched surface revealing both a fully formed, carpeted region alongside developing crystalline plates with a few attached fibrillar scales, we conclude the fibrillar scale layer may have been dislodged. Furthermore, if the fibrillar scales were retained during division, we would expect to see more cell surfaces with a complete carpet than we do; those present may represent the few cells

Figs. 17–20. P and E fracture faces of the PM of *H. brunnescens*

Figs. 17 and 18. P fracture faces of the PM reflect the exact position of the overlying plates. The concentration of IMPs located beneath overlying plates is high. That region of the PM corresponding to the overlying plate borders is slightly depressed towards the cell cytoplasm and relatively devoid of IMPs (arrows). The IMPs are in two compact rows (docking sites) in that part of the inner leaf that overlies an ejectisome (inset Fig. 18). Fig. 17 × 60,000; Fig. 18 × 95,000

Figs. 19 and 20. E fracture face of the PM. EF clearly reflects the presence of the hexagonal surface plates. A high concentration of IMPs separates in this leaf as well, although they are normally missing from those regions of the membrane corresponding to the outer plate borders. In Fig. 20 the IMPs appear to be in rows running in at least two directions (black lines) and equally distributed over the surface. Distinct membrane domains without IMPs and corresponding to overlying plate borders are not so obvious in this fracture, which may reflect a different stage in cell development. The bumps (arrows) correspond to the depressions in Fig. 16 and are related to the position of underlying ejectisome vesicles. Fig. 19 × 32,000; Fig. 20 × 60,000



Figs. 17–20

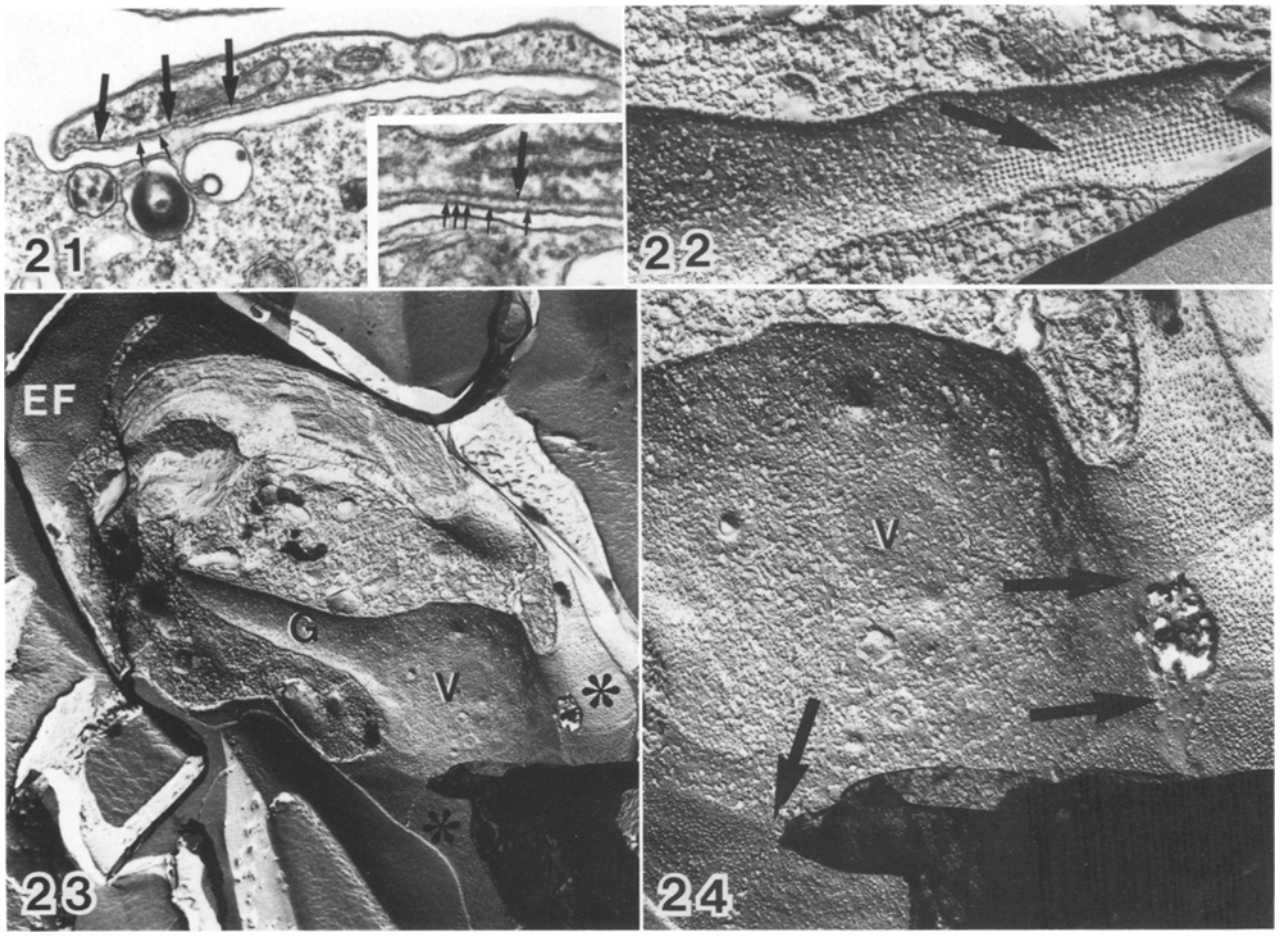


Fig. 21. Thin sections showing the gullet and gullet associated layer (large arrows) parallel to that portion ofthe tubular gullet membrane near the cell surface. Electron dense particles (small arrows, inset) often appear between the gullet membrane and this material. Fig. 21 × 30,000; Fig. 21 (inset). × 42,500

Fig. 22. P face of the gullet membrane showing the crystalline lattice of IMPs (arrow) that appears to occur only over the position of the tubular membrane located away from the center of the cell. × 45,000

Fig. 23. P-fracture face of the PM at three positions over the cell periphery; the region of the vestibulum (*V*), gullet (*G*) and that portion associated with the periplast (asterisk). The E face (*EF*) is observed at another region of the cell. × 60,000

Fig. 24. Region of the vestibulum seen in Fig. 23 at higher magnification. The texture of the P face is rougher in the vestibulum and the concentration of IMPs is less. The point where the overlying plates end at the edge of the vestibulum (arrows) is clearly visible. × 30,000

that did not divide during that cycle. Perhaps a reorganization of all crystalline plates is required following division, before or during which fibrillar scales are dislodged.

Although the basic components of the cryptomonad periplast seem to be present throughout the class, the morphology of the IPC and SPC and their relationship to the PM can vary between different species (SANTORE 1977, 1982 a, b, 1984, BRETT and WETHERBEE 1986) and even within the same species (HILL and WETHERBEE 1986). Previous freeze-fracture work has described the substructure of the cryptomonad PM, including the distinct rows of IMPs that correlate precisely with, and act as attachment sites for, the plates of the IPC (GANTT 1971, HAUSMANN and WALZ 1979, BRETT and WETHERBEE 1986). In some cryptomonads the IPC can be dominant, with thick, distinct plates being found just beneath the PM and attached to it at precise points (GANTT 1971, FAUST 1974, BRETT and WETHERBEE 1986); the IPC may then be isolated from sonicated cells and its structure revealed on whole mounts by negative staining (GANTT 1971, FAUST 1974). In one case, the IPC was shown to be a crystalline lattice (FAUST 1974). Unlike cells with a distinct IPC, the exact structure and position of the IPC in *H. brunnescens* is hard to ascertain (see also SANTORE 1982 a), as this layer is both

thin and tightly appressed to the undersurface of the PM. It has been reported that the IPC consists of plates that mirror the surface plates (SANTORE 1982a), although our observations indicate the IPC forms a continuous sheet that is absent only at the vestibulum/gullet region and where ejectisomes underly the PM. Fracture faces suggest that the relationship of the IPC (as well as the SPC) to the PM alters at the plate borders, and the IPC may be either absent or associated with the PM in a different manner in these regions. Whatever its configuration, the IPC undoubtedly acts as a form of barrier, preventing the fusion of membrane-bound vesicles with the PM and the consequent deposition of materials on the surface. However, as subunits plus fibrillar scales (or their precursors) require access to the surface during plate development, macromolecules should be able to reach the PM by passing through the IPC. Access to the surface may then be gained at the plate boundaries, as the distribution of IMPs shows two distinct membrane domains within the periplast-associated PM; the region of PM corresponding to the overlying plate borders lacks IMPs and may be more fluid and accessible. The concentration of IMPs under the crystalline plates is very dense, and access to the surface would be less likely through these more stable positions. The structure of the plate borders during development and their apparent relationship to the formation and distribution of fibrillar scales during "carpet" formation also suggests that these components may reach the surface at the plate borders.

In some Prasinophytes (*e.g., Pyramimonas*, MOESTRUP and WALNE 1979) up to six different scale morphologies may be found in tiers on both the cell surface and flagella. All scale types are synthesized by the Golgi and assembled into layers in the scale reservoir, a large vacuole located near the surface and continuous with the PM. Once formed, the scale groups migrate out of the reservoir at a single region near the flagellar bases and move over the surface (MCFADDEN and WETHERBEE 1985). *H. brunnescens*, like many other cryptomonads, has a vestibulum and gullet near the flagellar bases that is free of the normal periplast components. These regions could conceivably provide access to the surface for subunits, entire plates or scales to be synthesized and assembled within the cytoplasm, arranged into a larger complex and then secreted and deployed over the surface. Associated with the vestibulum in most species is a single Golgi stack from which vesicles may fuse with the PM in this region. However, there is no evidence that the SPCs described here arise at a single point (*e.g.*, the vestibulum and/or gullet) and then migrate onto the surface. The surface components of these regions are different from those on the surface. In addition, the microarchitecture of the PM indicates its involvement in periplast formation. The substructure of the PM in other scale-bearing algae does not exhibit the same stable membrane domains seen in *H. brunnescens*.

Ejectisomes are occasionally observed in *H. brunnescens*, although they often discharge during fixation or freezing. Their association with the PM and periplast components has therefore not been studied extensively, but is best observed in those species where periplast associated ejectisomes are numerous and do not readily discharge. Three studies have already appeared (see HAUSMANN and WALZ 1979, GRIM and STAEHELIN 1984, BRETT and WETHERBEE 1986).

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