**EUGLENA PLASMA MEMBRANE DURING NORMAL AND VITAMIN B12 STARVATION GROWTH**

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**SUMMARY**

Freeze-fracture and optical diffraction techniques were used to study the organization of the *Euglena* pellicle during the normal and replicative stages of the cell cycle and during vitamin B12 starvation. It was shown that the diffuse layer underlying the tripartite structure has a fibrillar structure. Despite the absence of homology in the 2 fracture faces of the pellicle, the EF striated and the PF particulate ones appear complementary as shown by optical diffraction studies; it must therefore be considered as a true membrane. The grooves are free from such particles and striations. They appear as a specific pattern of the cortex, different from the ridges in their structural organization and their replicative capacity as observed during vitamin B12 starvation. This notion is confirmed by the mode of pellicular growth which is characterized by 2 steps. The first occurs during the early replicative stage (pre-mitotic phase of the cell cycle) when the formation of a new ridge is correlated with the appearance of the 'minor' orientation of a 2-dimensional lattice on the EF and the PF faces and the spread of the particles over the PF face of the space between the old ridges. The second takes place during the lengthening of the ridges from the initiating posterior side (non-replicative stage). During this second step, the 'major' orientation of the lattice is preferentially observed in control cells and exclusively in starved cells.

The striking differences between the grooves and the ridges is discussed, as well as the 2 modes of growth and their significance in morphogenesis.

**INTRODUCTION**

Like most Euglenideae, *Euglena gracilis*, both normal and vitamin B12 starved, possesses an outer cell wall called the 'pellicle' which has aroused interest since Stein (1878) first observed it as a striated external layer. The peculiar ridge-groove shape of the Euglenoid surface has often been described (Groupé, 1947; Gibbs, 1960; Kirk & Juniper, 1964; Buetow, 1968; Guttman & Ziegler, 1974) and the semiconservative inheritance of the strips has been clearly demonstrated (Sommer & Blum, 1964; Leedale, 1967; Hofmann & Bouck, 1976).

The structural organization of this cell membrane complex has also been analysed by the freeze-fracture method (Holt & Stern, 1970; Schwelitz, Evans, Mollenhauer & Dilley, 1970). These authors obtained 2 different fracture faces: one, EF, striated and the other, PF, particulate. While Schwelitz *et al.* suggested that the striated one was the most internal, Holt & Stern placed this layer immediately under the outermost diffuse mucilage. It was not until 1972 that Walne, Aldrich, Bartlett & Pendland correctly interpreted the organization of the non-complementary striated and particu-
late fracture faces. In a recent paper Miller & Miller (1978) indicated that this non-complementarity may be the result of a post-fracture deformation in one of the faces.

During B12 starvation of *Euglena gracilis*, cell divisions stop while RNA and protein synthesis carry on; the cell volume increases up to 10-fold more than a plateau stage cell. We recently demonstrated that the correlated increase in cell surface occurs by a 2- to 3-fold lengthening of the parental ridges, without any modification of the grooves (Brè & Lefort-Tran, 1978). The normal growth, called 'intussusceptive' by Hofmann & Bouck (1976), is blocked and is only reinitiated by vitamin B12 refeeding.

We have been interested in the modifications induced during such growth at the level of the molecular organization of the cell membrane complex compared to the structure in normal growth.

In this study, we compare the molecular organization of control and starved cells with the help of freeze-fracture and optical diffraction techniques. We have been able to show that the E striated and P particulate faces of the ridge are indeed complementary and show the same 2-dimensional lattice. The grooves, which do not display such organization during the non-replicative stage, take on new characteristics during intussusceptive growth. These features are compared to the striking modifications observed in ridges and grooves during growth after withdrawal of vitamin B12 in order to explain their different responses to vitamin B12 starvation.

**MATERIALS AND METHODS**

Cells of *Euglena gracilis* Z (strain 1220 Cambridge) were grown in mixotrophic medium with glutamate–malate (Greenblatt & Schiff, 1959) or DL-lactate as carbon source (33 mM) or in autotrophic conditions on the same mineral medium under a 2000-lux continuous illumination with vitamin B12 (2 μg/l).

For starved cultures the same medium was used but the vitamin B12 was omitted.

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**Figs. 1-4. Control cells. Non-replicating stage.**

- Fig. 1. Section perpendicular to the cortex. Ridges (r) and grooves (g) have fairly constant dimensions. Note a dense, irregular material, believed to be mucilage (mu) exterior to the plasma membrane (pm). A dense layer (dl) underlies the plasma membrane. Four microtubules (mt) are observed in each ridge and one cisterna of endoplasmic reticulum (er). × 108,000.

- Fig. 2. Tangential section to the strips. Note alternating ridges (r) with undulating (er) cisternae which runs parallel to the strip. Microtubules (mt, 1, 2, 3, 4, 5) are visible, corresponding to those seen in transverse sections. The dense layer (dl) appears as regular striations at the groove level and as thin fibres in the ridges (arrows). × 93,000.

- Fig. 3. Negative staining (phosphotungstic acid 1%). Interior surface of isolated pellicle. The grooves are folded (g) and the ridges (r) (concave surface) are filled with fuzzy material which appears regularly striated (arrows), with the same periodicity as in Figs. 2, 4; mt, microtubules. × 54,000.

- Fig. 4. Freeze-etching. The fractured plasma membrane exposes the E striated face of the ridge (r) and the smooth face of the groove (g). Overlying the strips is a fibrous material corresponding to the dense layer (dl) which appears after etching. It is composed of twisted fibres of about 10 nm diameter with a periodicity of about 20 nm (arrows). In the concave ridges the subunits of the fibres appear. × 54,000.
Normal and B\textsubscript{12} starved Euglena membrane
Freeze-etching

The cells were spun, fixed in 2 % glutaraldehyde in 0·1 M phosphate buffer, pH 6·8, for 1 h at room temperature. After washing, the cells were dispersed in glycerol to a concentration of 30 % in the same buffer (v/v). Samples were frozen in Freon 22 (conventional drop technique), then transferred to liquid nitrogen.

In some preparations, the cells were broken in a French press (2000 psi, 1·38 x 10^4 kN m^-2) in 0·05 M tricine buffer, pH 7·6, with 0·2 M sucrose and 10 mM NaCl. By differential centrifugations, the pellet enriched by pellicles was treated as above or frozen in the same buffer without sucrose or glycerol by the spray-freezing method according to Bachmann & Schmitt (1971).

Replicas were prepared in a Balzers BA 360M apparatus at −100 °C with or without etching depending on the glycerol treatment. They were observed in a Hitachi Hu 12A electron microscope at 75 kV.

Thin sections

The cells were centrifuged and 2 % glutaraldehyde in 0·1 M phosphate buffer, pH 6·8 was added to the pellet for 2 h at room temperature. The fixative was removed by washing, the cells were postfixed for 1 h at 4 °C in 1 % osmium tetroxide in the same buffer, dehydrated in ethanol and embedded in Epon Araldite. Sections were stained with uranyl acetate and lead citrate.

Optical diffraction patterns

These were recorded at the focal plane (focal length 1200 mm), with a lensless reflex camera on Ilford FP4 film using a He-Ne laser (wavelength 632·8 nm) for illumination. In the illustrations the diffractograms are seen oriented perpendicular to the corresponding striations of the freeze-fracture micrographs.
Normal and $B_{12}$ starved Euglena membrane
RESULTS

As previously described (Leedale, 1964; Hofmann & Bouck, 1976), the pellicle of both normal and starved cells (Bré & Lefort-Tran, 1978) appears in sections (Figs. 1, 20) as composed from the exterior to the interior of 2 dense layers with a clear space between them. The tripartite structure measures around 10 nm. Just beneath the inner part of this membranous structure is a diffuse electron-dense region extending up to 18 nm. When isolated pellicles are obtained by cell disruption, this material called the ‘submembranous layer’ by Hofmann & Bouck (1976), is still present. In negatively stained preparations it appears as a fuzzy material between the strips of the pellicle, more abundant in the thickness of the ridges, as seen from the interior of the cell (Fig. 3). Sometimes, in these preparations (arrows, Fig. 3) some regularly ordered transverse fibrillar material with a mean periodicity of 17 nm can be observed. In freeze-etch preparations with deep-etching (Fig. 4), the fibrillar structure of this region appears clearly, composed of rough fibres, about 10 nm in diameter with a periodicity of about 20 nm, which lie at right angles to the main axis of the strips and seem firmly attached to the triple structure of the plasma membrane; the elementary fibres seem tightly twisted in the depth of the grooves whereas they unfold in the ridges (see legend to Fig. 4). It is sometimes possible to see microtubules lying adjacent (Fig. 20, p. 257). In tangential sections (Fig. 2), these twisted fibres, with the same periodicity, appear more clearly in the posterior angle of the groove where one or two microtubules can be seen embedded (microtubule 2 or 3, Fig. 2). The unfolded thin fibres also appear at ridge level in this picture (arrow, Fig. 2, see also Fig. 20). The 3 types of preparations (negative staining, freeze-etch and sections) definitely demonstrate that this layer, which appears diffuse in sections and tightly linked to the cellular membrane, has a composite fibrillar structure which could play a role in cellular movements and probably has a specific chemical composition as will be discussed later.


Fig. 9. Alternating grooves (g) and ridges (r) showing the P particulate face (PF) and the E striated one (EF). x 55,600.

Fig. 10. Conventional drop technique. P particulate face (PF) of the ridge (r). Note that the groove (g) is devoid of particles (arrow). x 54,000.

Fig. 11. Conventional drop technique with etching. In this replicating stage, the space between 2 ridges enlarges and is covered by particles. The bulging space between old ridges (r) is the first indication of a new strip (nr). The true smooth outer surface of the pellicle is revealed by etching (EOS). x 54,000.

Fig. 12. Conventional drop technique. PF, particulate fracture face of the plasma membrane. The narrow grooves (g) are free of particles. r, ridges; in this preparation, some of the particles are close together and form rows or striations (arrows) with the major orientation (see Discussion). x 48,600.

Fig. 13. a. PF fracture face. The packed distributed particles (4000/μm²) display the 2 orientations illustrated by Fig. 13b. x 46,200. b. Optical diffraction diagram on the PF face reveals the presence of a 2-dimensional reciprocal lattice identical to that shown in Fig. 6a on the EF face. M, major orientation.
Normal and $B_{12}$ starved Euglena membrane
Control cells

In control cells, after freeze fracturing, the tripartite membrane structure appears split into 2 parts, as previously described (Schwelitz et al. 1970; Walne et al. 1972; Miller & Miller, 1978): the outer, concave EF face has regular striations and the inner convex PF face is particulate (Figs. 9, 20).

Control EF fracture face. In normal cells, during the non-replicative stage of the cell cycle (\(G_1\)-S-G2) the ridges of the pellicle have the same regular size (Hofmann & Bouck, 1976; Bré & Lefort-Tran, 1978) (Figs. 1, 5). After freeze fracture the EF fracture face appears with a regular ordered pattern of ‘major’ striations which are spaced at around 6-3 nm and oriented at an angle of about 40° to the direction of the strips (Figs. 5, 6). A rough striation (arrows, Fig. 6), with the same orientation, also appears, sometimes with a repetitive spacing of 32-0 ± 5-0 nm. In some preparations it is possible to observe a discrete and wider ‘minor’ striation which is better seen on the diffractograms.

The optical transform analysis of the EF control fracture face shows a 2-dimensional lattice. In Fig. 6B, the major points measured on the lattice are distributed along 2 axes, with repetitive spacings of around 6-3 nm/37° (‘major’ striation) and 8-0 nm/57° (‘minor’ striation) respectively corresponding to the orientations and spacings observed on the freeze-fracture preparations. Both the values of the minor and major striation angles, using the direction of the groove as the reference axis, must differ with the curvature of the ridge. For simplicity, we shall use the terms major and minor for the 2 orientations of the lattice.

In some favourable pictures, the continuity between ridges and grooves is observed (Fig. 5); the grooves of cells in \(G_1\) phase of the cell cycle appear completely devoid of striations. This remarkable difference between ridges and grooves vanishes as the cell enters the replicative phase of the cycle (M) (Figs. 7, 8). In this case, the space between adjacent ridges (\(r\)) increases and is organized in a pattern in which the minor orientation shows up fairly well (Figs. 7B, 8B). In one part of the ridge which is considered as the old one (or), the optical diffraction diagrams given in Figs. 7C and 8C show the same pattern as Fig. 6, with the 2 directions the major one being more apparent. However, in the growing zones of the pellicular strips (nr) there is developed a system of apparent striations which occur at an angle to the direction of the ridge, corresponding to minor direction. The major striations are weak but still present (Figs. 7B, 8B).

Control PF fracture face. The PF convex particulate face of the ridges (Figs. 10–13) appears with apparent randomly distributed particles of about 10.0 nm in diameter, and with a density of around 4000 particles/\(\mu m^2\). They are more densely packed than in other cell membranes studied. Again, as for the EF fracture face, the groove is devoid of particles (Figs. 9, 10, 12, 13A) and shows a quite different organization. As the strips are replicated, the width of the space between adjacent ridges increases and becomes covered with particles (Fig. 11). This region is the site of formation of new strips, as shown in sections of replicating pellicles (Bre & Lefort-Tran, 1978). The optical diffraction pattern (Fig. 13B) of this particulate fracture face (PF) is very

Fig. 14. A, depending on the area on the E fracture face, the 2 types of striations are more or less evident, as one can see in optical diffraction patterns B and C respectively. x 60,700.

Fig. 15. A. This picture summarizes the main features of the starved pellicle. On the left a ridge is shown; the apparent striation of this corresponds to the major orientation. Note the absence of striations on the groove (g) (compare with Fig. 5). On the right of the picture, a large ridge is seen, built during starvation, which possesses the major apparent striation; arrows indicate defects in the lattice which must be compared to Figs. 16, 17. x 54,000. B. The optical diagram of the area outlined in Fig. 15A shows only the major orientation (M) of the lattice; the repeat distance of the spots on the diffractogram is half of that shown in Fig. 6B for the control, indicating doubling of the periodicity.
characteristic of a 2-dimensional lattice with the same parameters as the pattern obtained on the EF fracture face, showing the exact complementarity of E and P faces of the *Euglena* cortical membrane.

**Starved cells**

As previously demonstrated (Bre & Lefort-Tran, 1978), during vitamin B₁₂ starvation, the intussuceptive growth of the cortex is blocked: the cellular surface increases by exclusive lengthening of parental ridges. When moderately starved cells are observed one can find, as in control cells, a striated pattern of the EF fracture face with a 2-dimensional lattice (Fig. 14A, B). In some cases, depending on the area, the intensity of the reflexions of the minor direction are weak but present (Fig. 14C).

In starved cells in which divisions have been blocked for 6 days, the ridges are twice as wide as the control ones (Fig. 15A). The rough striations are closer than in normal cells, i.e. 15.4 nm instead of about 40.0 nm, and some defects appear (arrows, Fig. 15A). Optical diffraction shows one major direction only (Fig. 15B), with a first order of 14.4 nm, which is double that shown in Fig. 6A for control cells (6.3 nm) and which corresponds more or less to the interval measured on the picture, indicating a submolecular modification of the ridge architecture. This change becomes more striking in some samples (Figs. 16, 17) in which the regular oriented striation disappears. In its place, patches of striated areas are formed (Fig. 16), with different orientations intermingled with more or less particulate regions. In diffraction patterns derived from such an area the 2 directions can still be seen, although the secondary one may be weak.

This apparent disorder can be compared to that seen in pellicle structures observed after reinitiation of cellular divisions by renewal of vitamin B₁₂ in the growth medium (Bré & Lefort-Tran, 1978) (Figs. 17, 18).

Fig. 18 shows a regular succession of large (l), medium (m) and small (s) ridges which have been previously discussed and interpreted as a differential growth of the ridges during starvation (Bre & Lefort-Tran, 1978). On the old, large ridges, a
depression can be observed (Fig. 18, arrow) which is always present and which was first interpreted as a defect or artifact induced by the preparation procedure. In the corresponding freeze-etch preparation (Fig. 17), these depressions are again found (arrow) and appear as areas devoid of striation but with an irregular distribution of particles. We believe that these regions are not preparative artifacts but show the first stages in formation of a strip which did not progress because of the $B_{12}$-starvation block. The PF fracture face (Fig. 19) of the starved cell pellicle has particles of the same size and density ($4075$ particles/$\mu$m$^2$) as the PF control cells; instead of having a convex appearance, this face often looks shrunken and in this aspect matches well with the irregular appearance of the EF face. Despite this apparent anarchic surface, a distribution of particles along the main axis can be seen.

**DISCUSSION**

The freeze-fracture technique lends itself to study the internal organization of the membrane since the fracture plane lies within the lipid bilayer (Branton, 1966; Pinto da Silva & Branton, 1970) and exposes the inner faces of the membrane which usually exhibit almost complete complementarity.

In the present study on the cortical complex of *Euglena*, optical diffraction analysis has been used in conjunction with the freeze-fracture technique and enables us (a) to obtain new information on the diffuse layer; (b) to demonstrate the complementarity of the 2 halves of the tripartite structure despite their striking differences; (c) to show the remarkably different organization of ridge and groove membranes during the non-replicative stage; (d) to show the transformation in the membrane during intus-susceptive growth; and (e) to discuss the modification of such events and their relation with the block of division during $B_{12}$ starvation.

The Euglenoid genera possess a complex cortical organization which has been misunderstood until very recently. Characterized by a series of helicoidal strips (grooves and ridges) which run from the gullet to the posterior end of the cell, the cell membrane complex, called the pellicle, maintains this architecture during changes in cell shape, cell division and even after disruption and extraction.

In fact, the pellicle of *Euglena* is composed of 2 constituents, an external tripartite structure 8–12 nm thick as previously described by several authors (Gibbs, 1960; Kirk & Juniper, 1964; Schwelitz et al., 1970) and a diffuse electron-dense layer 13–18 nm immediately beneath.

Leedale (1964) suggested the existence of this layer and this was confirmed by Kirk & Juniper (1964). Schwelitz et al. (1970) were able to find it again in transverse and tangential sections and to show its striated structure. Unfortunately, the latter authors confused this layer with the outermost half of the plasma membrane. Our micrographs indicate that this diffuse layer which can most readily be demonstrated in tangential sections, in negatively stained and freeze-etch preparations, lies just beneath the plasma membrane along the ridges and the grooves. It is thicker in the notch of the ridges, as also appears in isolated pellicles (Hofmann & Bouck, 1976).

In our deep-etch preparations this layer consists of rough twisted fibres with a
Normal and B₁₂ starved Euglena membrane

diameter of from 10 to 15 nm which are at right angles to the main axis of the strips. Their dimensions, structure and position suggest that they could play a role in the contraction and expansion of the cell and in retaining the ridge and trough organization, even after disruption. In their work, Kirk & Juniper (1964) suggested that this layer could be a protein of the actomyosin type. Immunological tests would solve this intriguing problem.

Fig. 20. Drawing of the pellicular cortex of Euglena gracilis. Ridge (r) and groove (g) form a repetitive unit. The plasma membrane (PM) limits the cell with 2 black lines separated by a clear space. Just below is the dense fibrillar layer (dl). The microtubules (mt) are more-or-less parallel to the axis of the ridges; numbers 2 and 3 are embedded in the dense layer. er, endoplasmic reticulum. By freeze-fracture the plasma membrane gives 2 complementary EF and PF faces: EF is the exoplasmic fracture, with a bidimensional lattice, PF is the particulate protoplasmic fracture. The left part of the drawing exhibits half of the fractured plasma membrane (EF fracture), and the fibrils of the dense layer (dl) disclosed by etching. The outer surface of the plasma membrane (EOS) is smooth. The arrows indicate the anterior end of the cell.

The outer cortical membrane, which overlies the alternative ridges and grooves, is around 10-15 nm wide and appears, with the conventional technique, as a tripartite structure in transverse sections and splits as usually by freeze fracture, into 2 halves (a) an outer E fracture face, highly organized in a regular pattern which gives an optical diffraction diagram of a 2-dimensional lattice as shown by Miller & Miller (1978); and (b) a P inner particulate fracture face (Walne et al. 1972; Miller & Miller, 1978 and our present results) (Fig. 20).

Because of the apparent non-complementarity of the 2 fracture faces and the unusual high protein content of the cortical complex (Hofmann & Bouck, 1976), the membranous character of this tripartite structure has until now been doubted.
Walne et al. (1972) did not solve the problem, while Miller & Miller (1978) believe that the particles are artifacts and the non-complementarity results from changes induced on the P face by the freeze-fracture process. According to Miller, some apparent complementarity can be obtained when the fracturing process is performed below $-150^\circ C$.

Using the conventional drop technique, instead of spread particles, we have observed discrete striations of this type on the P face, but they seem to be unrelated to the fracturing temperature process and we believe that they are artifactual. Because of the high speed of freezing, rapid cryofixation methods, such as spray-freezing (Bachman & Schmitt, 1971) or sandwich-freezing (Gulik et al. 1977), minimize the artifacts as we showed previously (Lefort-Tran et al. 1978). The use of such methods in the course of this work has always enabled us to reveal the P fracture face with distinct particles more or less randomly distributed. The optical diffraction results clearly show that the particles are, in fact, fairly well organized in a 2-dimensional lattice whose quantitative parameters are identical to those obtained for the E face. In our opinion, these results confirm the true complementarity of E and P faces and the membranous character of the tripartite structure.

In the course of this study we have shown that the organization of the ridges and the grooves is quite different. The latter are devoid of regular striations on the E faces and of particles on the P faces during the non-replicative stage. These differences in the cortical membrane of ridges and grooves diminish as the replication of the strips occurs. These observations on the pellicle of stationary and replicating cells are to be compared to the description of the gullet and transitional region of the membrane given by Miller & Miller (1978) and also observed in our preparations. Near the neck of the gullet, the ridges are clearly identified with particles on the P face and the regular striated E face. Deep in the cell, the contour of the ridges becomes less pronounced and flattens out. The differences between ridges and grooves disappear. Normally localized exclusively on the ridges, the particles become randomly distributed all over the P face as for most cell membranes. These features are, in some ways, to be compared with the description given for the oral cavity of *Tetrahymena pyriformis* (Sattler & Staehelin, 1979) and give us another reason for thinking that the particles of the P face on the ridges of *Euglena* are not artifacts.

The knowledge of the striking structural difference between ridge and groove membranes may help towards a better understanding of normal growth and growth under conditions of B$_{12}$ starvation.

From our results, we suggest that the normal growth of the pellicle could be divided into 2 steps: (i) During the replicative stage, a new ridge is initiated at the posterior margin of the old one. At membrane level, we observe that the space between 2 ridges enlarges and is covered by particles on the P faces and by a regular pattern of striations with one preferential direction (the 'minor' direction) on the E face. (ii) During the non-replicative stage, the new ridge lengthens exclusively from the posterior to the anterior side as clearly demonstrated by the immunological studies of Hofmann & Bouck (1976). They show that labelled antigen sites on the surface of the *Euglena* cell membrane are localized on the posterior margin of the ridge. This could mean that
some antigenic determinants are localized exclusively on the posterior portion of the pellicle strip or that extension of the ridge occurs without diffusion of the specific antigenic sites. As the small bud grows the major striation becomes more and more apparent in the adult ridges, and the E face displays the characteristic regular striation with 2 orientations as demonstrated by optical diffraction.

The problem of how a new groove is built between the posterior side of the old ridge and the new one still remains unsolved.

During vitamin B$\text{_{12}}$ starvation, intussuceptive growth is blocked. The increase in cell surface occurs exclusively by a lengthening of the parental ridges from the posterior margin, as was discussed by Bre & Lefort-Tran (1978). From optical transforms of this region we obtain a 2-dimensional lattice corresponding to the zone built before starvation. As in control cells, growth progresses anteriorly. In the growing regions of starved cells, the diffraction pattern gives only one major orientation. Such growth areas are characterized by a molecular organization displaying a regular major striation at an angle of about $34^\circ$ to the axis of the ridge. The minor striation, which seems characteristic of the replicative stage is practically non-existent. On the other hand the spacings between striations are twice those of the control pellicle.

The data thus indicate that there are 2 classes of components defined by their integration in the 2-dimensional lattice of the cortex. The first one is implicated in the organization of the minor orientation and is synthesized exclusively during the replication of the pellicle; the second one which contributes to the major orientation is synthesized during both the replicative and non-replicative phases. During B$_{12}$ starvation the lengthening of the pellicle seems exclusively related to the incorporation of this component.

The cortical complex of Euglena displays a highly repetitive structural pattern which is closely comparable to the cortex of ciliates such as Paramecium (Sonneborn, 1974) and Tetrahymena (Elliott, 1973; Sattler & Staehelin, 1979). From our results it appears that each component of the pattern—the grooves and the ridges—has its own structural organization of the plasma membrane, probably a specific chemical composition, its individual response to cytoplasmic and/or nuclear signals, and a characteristic replicative capacity which can be modulated by external factors such as vitamin B$_{12}$ starvation.

At the cellular level, morphogenesis takes place either during division or during the development of new structural and physiological properties. In both cases, it involves a cycle of changes in the organization and physicochemical properties of the surface and in the location, shape and size of organelles, particularly of the nucleus. Previous data and speculations (Pardee, 1971; Bennet, Boyse & Old, 1972; Beisson, 1972; Sonneborn, 1974; Ruiz, Adoutte, Rossignol & Beisson, 1976) draw attention to this correlation and suggest that it might be the changes in the surface, in contact with the environment, which trigger and control the internal changes.

The plasma membrane of Euglena is an example of such possible correlation. In this cell, during the $G_{1}$–S phase of the cell cycle, specialized organization and growth occur which are unidirectional from the initial posterior ridge of the crest. During the pre-
mitotic phase, while the nucleus leaves its central position to migrate behind the gullet, the pellicular pattern is replicated. A young ridge is initiated between 2 old ones and at the membrane level, striking changes are observed as previously discussed. By vitamin B₁₂ starvation, the cell cycle is retained in S-G₂ phase. The nucleus keeps its central location and does not undergo normal division; simultaneously, the intussusceptive growth of the pellicle is blocked. The growth of the pellicle continues by lengthening of the ridge from the posterior side. The molecular organization of the membrane is defective in at least one component which is synthesized and integrated in the membrane during a short period of the cell cycle. The addition of vitamin B₁₂ induces normal intussusceptive growth of the pellicle and nuclear migration and fission. The 2 events are strictly contemporary and require specific protein synthesis which can be blocked by cycloheximide (in preparation).

If our conclusions are valid, it may even mean that the absence of vitamin B₁₂ in the medium prevents the synthesis or the correct integration of a specific membrane protein involved in the replication of pellicle.

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